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EXPERIMENTAL STUDIES WITH MUSCICIDES AND OTHER FLY-DESTROYING AGENCIES

By

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AND

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EXPERIMENTAL STUDIES WITH MUSCICIDES AND OTHER FLY DESTROYING AGENCIES.[1]

By Earle B. Phelps, *Professor of Chemistry*, and Albert F. Stevenson, *Sanitary Chemist*.

INTRODUCTION.

In the general public health campaign for the eradication of the fly, not the least important of the many destructive measures available are those capable of being employed within the household. These constitute in a measure the last line of defense and are aimed against those flies, relatively few in number, which have escaped such general public measures as the elimination of breeding places and that most important individual measure, effective screening. Even when most successful these measures have not heretofore given entire protection, and it has been necessary to supplement them with one or another form of destruction within the household itself.

For this purpose various devices are available, comprising in general poisoning, trapping, and "swatting." Each of these various methods has distinct advantages and disadvantages. The trapping of flies, either in mechanical traps or upon sticky preparations, while reasonably effective, is, under most conditions, an undesirable procedure by reason of its unsightliness and other unpleasant aspects. The practice of swatting, despite the faulty biological reasoning so often urged by its enthusiastic supporters, whereby the effectiveness of a single swat is multiplied many million fold, certainly does possess the advantage of a very definite 100 per cent efficiency. Its disadvantage lies chiefly in the effort and earnestness which it demands, factors which are apt to be affected by rising temperature inversely as the multiplication rate among the flies. The poisoning of flies is most simple and least objectionable from an esthetic standpoint except for the single disadvantage that the dead flies are scattered about the rooms and require some further attention on the score of neatness. Everything considered, this method seems to possess the fewest disadvantages in proportion to its advantages, and were it not for the single fact that poison fly papers and preparations are quite generally known to contain arsenic or other strong poisonous material, there is no doubt that their use would be greatly extended.

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[1] Manuscript submitted for publication Aug. 4, 1916.

DANGER ATTENDING USE OF POISON FLY PAPERS.

The fact that such use is attended with no small danger, especially among young children, can not be overlooked, nor has it been. The magazine "Child Betterment and Social Welfare," a monthly published in the general interest of the child, collected, through the medium, it is understood, of press clippings only, evidence of 47 cases of arsenical poisoning from the accidental or ignorant use of poison fly preparations during the summer of 1914. The majority of these cases were among children, 34 of them being among those under 3 years of age. Many of the cases resulted fatally. It is stated editorially in a discussion of this danger—and it is believed the statement is correct—that there is such a similarity between the symptoms of arsenical poisoning among young children and of cholera infantum that many of the cases diagnosed as this more common disease of infancy were doubtless the result of arsenical poisoning. No less than 20 medical journals, including those of some of the leading State medical associations of the United States, have published within the past two years editorials commenting upon this matter.

These facts being thus rather widely distributed, it is safe to assume that their knowledge has acted in a considerable degree to deter the better informed from using preparations of this type. Quite apart from the accidental or ignorant misuse of these arsenical preparations, and a matter which does not seem to have attracted attention or comment, is the ready accessibility without restriction of considerable quantities of a dangerous poison capable of being put to criminal use. It is at least inconsistent that a quantity of arsenic or its compounds in the form of poisonous fly paper can be obtained by anyone, without any explanation or record, which could not in most States be obtained through the usual channels except upon the registration of the purchase in a permanent record.

NATURE OF PROBLEM TO BE STUDIED.

It has seemed quite desirable, therefore, to investigate this subject with special reference to the selection of some other substances which, under ordinary conditions of use and of accidental or ignorant misuse, would not be so dangerous to the health and lives of children and which at the same time would serve equally well, or better, for the destruction of flies. It also seemed desirable to consider at the same time the general subject of sticky fly papers, more especially with reference to the relative value of the two types of remedy and to the overcoming of obvious objections to the present form and manner of use.

In order to carry out such an investigation it was necessary at the outset to develop a general plan for the experimental determination of the value of any given muscicide. It was obvious that experiments of this sort could not be conducted in a haphazard way under the ordinary variable conditions existing in practice. On the other hand, it was quite important that the results should be applicable in practice and that the relative effects of these variations should be determined and embodied in any general conclusions. The problem, therefore, reduced itself to one of establishing an experimental technique in which the variable conditions were subject to exact control and by means of which each of these could be independently modified and its effect recorded.

The first and most important of these variables is the fly itself. A study of the available information upon the life habits of this insect led immediately to the belief, which was later amply confirmed, that in order to procure quantitative data this factor must be adequately controlled. Questions of age and of physiological activity and the effect upon the latter of such factors as temperature, light, and food supply, had to be recognized as independent variables for study. Again the matter of reaction between the fly and the muscicide plays an important part in the ultimate results. It is quite conceivable that a given substance which, if freely taken, would be extremely effective, might be quite unacceptable to the fly, or be taken only under stress of circumstances. A satisfactory muscicide, however, must compete in attractiveness with other food materials generally quite accessible in the household and must be at least unobjectionable. The possibility of compounding with an unobjectionable poison certain attractive agents had also to be considered.

It was determined, therefore, as a first step, to propagate flies, so that a suitable abundance of material might be available for experiment, this material being of uniform and definitely known history.

LIFE HISTORY OF FLY.

In order to propagate the house fly at will for experimental purposes, it is necessary to know something of its life history. The following condensed and summarized account is from "The House Fly, Disease Carrier," by L. O. Howard.

BREEDING PLACES.

The house fly will breed in almost any fermenting organic matter, but, by preference, lays its eggs on horse manure. Next to horse manure it is probably most attracted to human excreta.

EGGS.

The eggs are minute, glistening white, long ovoids, approximately one-sixth of an inch in length. They are laid in clusters, a female fly laying on the average about 120 eggs at one time. She may lay several times during her life. At midsummer temperature the egg covering splits, and the larva crawls out about eight hours later.

LARVÆ.

The young larva, popularly known as the maggot, tapers from the blunt round hinder end to the pointed head end. It is white in color and about 2 millimeters in length. It is extremely active, and burrows at once into the substance upon which the eggs are laid. The house-fly larva casts its skin twice during its development. The rate of growth depends upon the temperature. At midsummer temperature the period from the hatching to the first molt is approximately 24 hours; from the first molt to the second molt, 24 hours; and from the second molt to the transformation to the pupa, 72 hours; making the duration of larval life 5 days. The larvæ are very active and migrate with ease throughout the manure.[1] Just before the transformation into the pupal stage the larvæ get yellowish in color, owing to the proliferation of fat cells in great numbers in anticipation of the resting, nonfeeding, pupal stage.

PUPÆ.

Before the transformation to the pupa the full-grown larva empties its alimentary canal and contracts from its own skin, the skin itself forming a nearly cylindrical pupal case about 6 millimeters in length. At first the pupal skin is a pale yellow, but it soon changes to red and finally to a dark-chestnut color. The pupal stage lasts about five days, after which the adult fly emerges.

ADULT FLIES.

Flies of the same species often differ in size. This is dependent upon the temperature during the larval and pupal stages. After the fly emerges from the pupal shell it never increases in size. The limit of life of an adult fly in summer is approximately 21 days.

THE BREEDING OF FLIES.

Two distinct lots of flies were propagated for use in this work. (1) A great quantity of stock flies kept solely for breeding. (2) The experimental flies.

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[1] We have found that the larvæ even leave the manure at times. They have traveled in some instances more than 50 feet over a concrete floor, where they perished. This migration was finally stopped by placing the manure pans on a bed of wet sand.

STOCK FLIES.

The flies used throughout these experiments were ordinary house flies (*Musca domestica*). They were laboratory bred from about 200 individuals obtained from a tray of infested horse manure. A small room was given up to the keeping and raising of the stock supply. Pans of fresh horse manure were always kept standing in this room, serving as a medium for the hatching of eggs. Food was also supplied in the form of pans of moistened bread. The temperature of the breeding room was maintained between 30° and 35° C. Fly eggs were deposited on the manure and also on the moistened bread. Many more were laid on the bread than on the same quantity of manure. It was found that the flies would not develop beyond the larval stage on the moistened bread, and consequently these larvæ when three or four days old were transferred to the manure, where they developed to adult flies. Approximately 10 days were required to raise an adult fly from the egg. This time in some instances was as low as eight and a half days. As only one generation of flies will breed in a lot of manure, the trays were freshly filled after each hatch.

EXPERIMENTAL FLIES.

For the experimental work it was desired to use material as homogeneous as possible. To this end each experiment was conducted upon a single batch of flies of the same age and bred under the same conditions. The factors of age, breeding temperature, and other physical conditions were noted in each case and made a part of the record of the experiment. By working in this way upon homogeneous material of known history, it was not only possible to obtain consistent quantitative results, but the effect of age and breeding conditions could also be measured and discussed.

The flies actually used in the experimental work were therefore raised in cages where they could be kept isolated and under observation. These cages were made with wooden bottoms and sides, but with a wire screen top. They were 36 inches on a side. A door 6 inches high, hinged at the top, was constructed at the bottom of one side and extended the width of the cage. It was found necessary to have three such cages in order to keep a continuous supply of proper material on hand.

A galvanized iron pan 6 inches deep filled with fresh horse manure was exposed to the flies in the stock-breeding room for 36 hours. It was then removed and placed in a breeding cage, where it remained until 2,000 or more flies hatched, when it was removed. Generally a plentiful supply of flies hatched within 36 hours from their first appearance. The mid-point of this period was taken as the "date of hatching." This procedure was regulated so that a brood of flies hatched every three days.

GENERAL PLAN OF EXPERIMENTS.

In this study it was desired to determine the relative efficiencies of fly poisons. For this purpose a standard solution of arsenic was used throughout as a control, and the poison to be tested was compared with this standard under identical conditions. Tests were conducted on a few substances which had been recommended as fly poisons and also on a number of more or less active inorganic and organic substances which are comparatively nonpoisonous to man. Certain supposedly attractive agents, such as milk, brown sugar, and molasses, were also studied in combination with some of the poisons, and a quantitative measure of their attractiveness thereby obtained.

Flies from the same batch and of known age and history were transferred to each of three experimental cages. These were exposed for a definite period to the poison to be tested, to the standard poison, and to water, respectively, and after 24 hours a count of the dead flies was made. From this was computed the crude mortality in percentage. The crude mortality was corrected in each case by the normal death rate for flies of this age and at this temperature as determined in the control experiments. These corrected mortalities for the poison and the arsenic standard were then used to compute a relative coefficient for the poison in question under the stated conditions of age and temperature.

EXPERIMENTAL CAGES.

Five identical experimental cages were used in this work. They were 36 by 36 by 30 inches and were made of wire screening with wooden floors. A wooden slide, 12 inches wide, which could be moved in a vertical direction, was inserted on one side to provide a means of access to the cage. Figure I shows the arrangement of the cages in the room with reference to illumination. All cages received as nearly equal illumination as possible.

CONTAINERS FOR POISON SOLUTION.

The solutions of poisons to be studied were placed in 6-inch white porcelain evaporating dishes, in which two pieces of filter paper were laid, one end in the solution and the other projecting about 3 inches beyond the edge of the dish. These papers remained wet throughout the test and served as a resting place for the flies.

TRANSFERRING AND COUNTING FLIES.

Flies were transferred from the breeding cage to the experimental cages and counted as follows: A hole about five-eighths of an inch in diameter was cut in one corner of the netting which served as a top for the breeding cages. This hole when not in use was plugged with a cork. A rug was thrown over the top of the cage so as to leave the hole just exposed. A 500 c. c. tincture mouth bottle was inverted over the hole and the flies, attracted by the light, passed into the bottle. One hundred or more flies will enter within two or three minutes. With the thumb placed over the mouth of the bottle these flies may be transferred to the experimental cage. If the thumb is then partially removed so that an opening is made just large enough for one fly to pass through at a time, the flies will leave the bottle at such a rate that they may be easily counted.

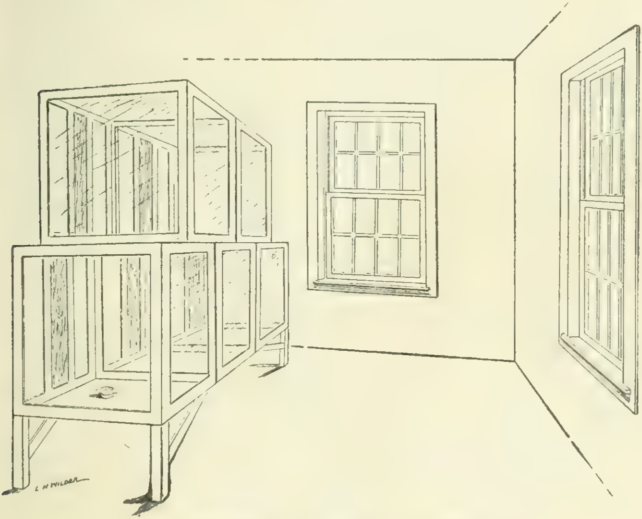

Fig. 1.—Arrangement of cages in experimental room.

STANDARD POISON SOLUTION.

Arsenic was chosen as the standard poison, because the majority of fly poisons on the market contain arsenic as the active ingredient. A series of solutions of varying concentrations of arsenic were tried, and a one-thousandth normal sodium arsenite solution was chosen as a standard, chiefly because it would kill about 50 per cent of the flies in a convenient time for laboratory tests.

The stock tenth normal arsenite solution was prepared by dissolving 4.95 grams of pure sublimed arsenious oxide (As_2O_3) and 20 grams of pure sodium carbonate in about 300 c. c. of distilled water by heating. When solution was complete, the liquid was cooled to

20° C. and the volume made up to 1,000 c. c. with distilled water. (Sutton, Volumetric Analysis, 8th ed., p. 149.)

Ten c. c. of the stock solution were diluted to 1,000 c. c. with distilled water. The resulting solution is one-thousandth normal arsenious oxide, containing 37.5 milligrams of arsenic per liter, and is referred to hereafter as the "standard arsenite solution."

PROCEDURE FOR TESTING KILLING POWER OF A MUSCICIDE.

One hundred flies of a known age were exposed in one of the experimental cages for four hours to 100 c. c. of the poison solution. At the same time and under the same conditions an equal number of flies were exposed in another cage to 100 c. c. of the standard arsenite solution. A third cage, containing 100 flies, together with moistened bread and water, served as a control. At the end of four hours the poisons were removed, and bread and water placed in the cages. Twenty hours from this time the dead flies were removed from each cage and counted.

It has been found that flies either do not eat or are very little affected by poison till after they are three days old. It is therefore best to start experimental work with flies at least four days old. Also flies more than 10 days old should not be used. for the normal death rate above that age is great.

Four hours was arbitrarily chosen as the standard time for exposing the flies to the poison. In the majority of cases this is insufficient to kill all the flies, so that quantitative results are obtained. The poison was then removed and an additional period of 20 hours allowed to elapse before making the count. It has been assumed that all flies affected by the poison would die within this time.

As temperature plays an important part in the killing of flies, it was kept as nearly constant as possible during a test and always recorded. Most of our work was done at about 32° C.

COEFFICIENT.

The ratio of the corrected mortality rate with a given poison to that with the standard arsenite solution, both rates having been corrected by the normal death rate without poison, has been taken as the coefficient of that poison. This coefficient has been found to depend also upon the age of the flies and the temperature of the experiment.

EXPERIMENTAL RESULTS.

NORMAL MORTALITY RATE.

Before proceeding with the quantitative analysis of the experimental results, it was found desirable to obtain more exact data than were available upon the normal death rate of flies under the varying conditions of the experimental work. The literature consulted gives only average figures without specific reference to the environment and the age of the insects.

A control test was made, parallel with each of the poison tests, with flies of the same batch, exposed to a dish of water under the exact experimental conditions obtaining in the test of the poison. The numbers of flies dying in these control tests being small, there was likelihood of a considerable percentage of variation and corresponding error in the use of the control figures as corrections. On the other hand, the combined data of all the control tests are sufficient in amount to give a reasonably correct picture of the actual facts. It was decided, therefore, to utilize these data for a special study of the normal rate of dying among flies of various ages and at various temperatures and to apply this normal rate as a correction to each experiment rather than the individual rate determined with that particular experiment. By this procedure the correction actually used is based upon the results of all the controls and subject therefore to less experimental error than if the individual rate were used.

The results of all these control experiments have been brought together in Table I.

TABLE I.—*Normal mortality (per cent per 24 hours) of flies.*

Date.	Age.	Temperature.	Mortality per 24 hours.	Date.	Age.	Temperature.	Mortality per 24 hours.
1915.	*Days.*	°*C.*	*P. ct.*	1916.	*Days.*	°*C.*	*P. ct.*
Nov. 9	4	30–35	4	Mar. 18	9	30.9	11
Nov. 10	5	30–35	4	Apr. 4	7	34.2	9
Nov. 11	6	30–35	4	Apr. 5	8	33.7	10
Nov. 12	7	30–35	7	Apr. 6	5	30.9	5
Nov. 29	10	30–35	27	Apr. 7	6	32.0	16
Nov. 30	11	30–35	25	Apr. 8	7	33.1	7
Dec. 17	7	32.0	8	Apr. 17	7	26.5	0
				Apr. 18	8	24.2	6
1916.				May 9	4	23.2	2
Jan. 13	10	34.2	10	May 11	6	22.7	1
Jan. 22	6	33.1	1	May 19	6	20.5	1
Feb. 5	3	32.0	5	May 20	7	21.0	2
Feb. 28	8	27.5	9	Do	7	21.0	2
Feb. 29	9	32.0	23	Do	7	21.0	1
Mar. 17	8	30.9	15	Do	7	21.0	2
Do	8	30.9	10	May 23	10	20.0	13
Do	8	30.9	10	May 24	11	20.0	20
Do	8	30.9	3	May 25	4	21.0	1
Do	8	30.9	11	May 26	5	22.0	0
Mar. 18	9	30.9	10	May 27	6	23.2	4
Do	9	30.9	15	May 31	10	23.2	2
Do	9	30.9	3	Do	10	23.2	3
Do	9	30.9	10				

Table II gives the same results averaged in groups according to ages and temperatures.

Table II.—*Normal mortality (per cent per 24 hours) of flies—Average experimental value arranged according to ages and temperature.*

Temperature (°C.).	Age in days.									
	2	3	4	5	6	7	8	9	10	11
20–25	......	......	1	0	3	2	6	......	6	20
25–30	......	......	......	......	......	0	9	......	......	......
30–35	......	5	4	5	7	8	10	12	19	25

The determinations were not numerous enough to furnish entirely consistent results, even when averaged by small groups, but the progressive variation with increasing age and rising temperature is sufficiently well marked to justify further treatment in the line of smoothing out the experimental results. Had all the control values been obtained at the same temperature and age of fly, this further treatment would be obvious, namely, a direct averaging of the results. In the case in hand a similar treatment has been applied in the construction of a table of average or most probable values extending over the experimental ranges of temperature and age. This table is given as Table III below, and the values thus obtained have been used to correct all the crude mortality rates obtained in the experimental tests.

Table III.—*Normal mortality (per cent per 24 hours) of flies—Most probable values arranged according to ages and temperature.*

Temperature (°C.).	Age in days.									
	2	3	4	5	6	7	8	9	10	11
20–25	1	1	1	2	3	3	4	6	8	14
25–30	3	3	3	4	5	7	9	11	......	......
30–35	5	5	6	7	8	10	13	15	18	25

PRELIMINARY SURVEY OF POSSIBLE MUSCICIDES.

Among the various substances that have been recommended for this purpose, arsenic, quassia sirup, formaldehyde, and potassium dichromate seemed to justify further study. These are variously mentioned as suitable muscicides, but no definite quantitative values have been reported. In fact, quantitative measurements of the efficiency of muscicides do not seem to have been made previous to the present study. The quassi sirup is recommended by the United States Dispensatory and is compounded as follows:

Macerate during 24 hours 1,000 parts of quassia wood (chips) with 5,000 parts of water, then boil for half an hour, set aside for 24 hours, and press. Mix the liquid with 150 parts of molasses and evaporate to 200 parts. A weaker decoction of the quassia does not kill the flies. From this the fly water, or fly plate, is prepared as follows: Mix when needed and dispense without filtering 200 parts of sirup of quassia, 50 parts of alcohol, and 750 parts of water. It is used by moistening with the mixture a cloth or filtering paper on a plate.

According to Howard[1] formaldehyde was first recommended by C. H. Popenoe, of the Kansas Agricultural College, in 1903, who used a 4 per cent solution. Others have advised the use of solutions varying from a teaspoonful of formalin to a cup of water (approximately 0.5 per cent formaldehyde) to 10 per cent formaldehyde. All writers seem to agree that the formaldehyde solution is very rapid and effective in its action.

Dichromate has also been recommended without specific data either upon the proper strength or the relative efficiency.

In addition to these three substances, a number of chemical compounds were selected rather at random, with the idea of establishing promising leads by covering a considerable range of chemical types. This preliminary survey served at least to eliminate many chemical groups and indicated certain substances as worthy of further investigation. No attempt was made to make this list exhaustive, even of the larger groups of chemical substances.

The results of this preliminary study are given in Table IV, from which those substances marked with an asterisk were selected for more detailed study.

TABLE IV.—*Preliminary study of muscicides.*

Date.	Substance.	Concentration.		Temperature.	Age.	Mortality (crude).	Mortality (corrected).
1916.			*P. ct.*	*°C.*	*Days.*	*P. ct.*	*P. ct.*
Feb. 16	Aluminum chloride ($Al_2Cl_6.12H_2O$)	0.1 molar	4.8	29.8	5	32	*28
	Potassium alum ($Al_2(SO_4)3.K_2SO_4.24H_2O$)	do	9.5	29.8	5	17	13
	Barium chloride ($BaCl_2$)	do	2.1	29.8	5	4	0
	Borax ($Na_2B_4O_7.10H_2O$)	do	3.8	29.8	5	13	9
	Calcium chloride ($CaCl_2$)	do	1.1	29.8	5	2	0
17	Ferric chloride ($Fe_2Cl_6.12H_2O$)	do	5.4	33.1	6	18	10
	Lithium chloride ($LiCl$)	Molar	4.2	33.1	6	29	21
	Magnesium sulphate ($MgSO_4.7H_2O$)	0.1 molar	2.5	33.1	6	2	0
	Manganous sulphate ($MnSO_4.4H_2O$)	do	2.2	33.1	6	2	0
	Sodium benzoate ($NaC_7H_5O_2$)	do	1.4	33.1	6	29	*21
23	Sodium salicylate ($NaC_7H_5O_3$)	do	1.6	33.1	4	65	*59
	Sodium fluoride (NaF)	do	.4	33.1	4	81	*75
	Sodium citrate ($2Na_3C_6H_5O_7.11H_2O$)	do	7.1	33.1	4	31	*25
	Sodium formate ($NaCHO_2$)	do	.7	33.1	4	38	32
	Potassium iodide (KI)	do	1.7	33.1	4	39	*33
24	Potassium nitrate (KNO_3)	do	1.0	31.2	5	37	*30
	Potassium chlorate ($KClO_3$)	do	1.2	31.2	5	47	*40
	Sodium potassium tartrate ($NaKC_4H_4O_6.4H_2O$)	do	2.8	31.2	5	14	7
	Zinc chloride ($ZnCl_2$)	do	1.4	34.2	5	26	19
	Saccharine (C_6H_4 CO NH SO_2)	do	1.8	31.2	5	22	15

Table V gives results of further studies of the more promising substances, together with average coefficients. This work indicated three substances of decided value, namely, formaldehyde, sodium salicylate, and sodium fluoride. The first two of these in the concentrations used and quantities exposed would be practically harmless to man. Sodium fluoride, even in this dilution, would probably be corrosive when mixed with the hydrochloric acid of the stomach. It is not as toxic as arsenic, but would be distinctly dangerous for general household use. Therefore the formaldehyde and the sodium salicylate were chosen for a careful study under varying conditions of temperature, concentration, and other factors, with a view to their recommendation for general and especially for domestic use. The results of this more detailed study are also given in full in Table V.

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[1] Howard, L. O., The House Fly, Disease Carrier, p. 185.

TABLE V.—*Detailed study of various muscicides.*

ARSENITE SOLUTION, ONE THOUSANDTH NORMAL.

Date.	Age.	Temperature.	Mortality (crude).		Mortality (corrected).		Coefficient.
			Test.	Standard.	Test.	Standard.	
	Days.	°C	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
1915.							
Nov. 9	4	30-35	....	45	....	39	....
Nov. 10	5	30-35	....	38	....	31	....
Nov. 11	6	30-35	....	55	....	47	....
Nov. 22	3	30-35	....	25	....	20	....
Nov. 23	4	30-35	....	25	....	19	....
Nov. 26	7	30-35	....	56	....	46	....
Dec. 14	4	30.9	....	20	....	14	....
Dec. 15	5	30.9	....	27	....	20	....
Dec. 16	6	29.8	....	31	....	26	....
Dec. 17	7	32.0	....	43	....	33	....
1916.							
Jan. 7	4	33.1	....	41	....	35	....
Jan. 10	7	32.0	....	29	....	19	....
Jan. 11	8	29.8	....	44	....	35	....
Jan. 12	9	33.1	....	44	....	29	....
Jan. 20	4	30.9	....	28	....	22	....
Jan. 21	5	33.1	....	59	....	52	....
Feb. 4	2	30.9	....	10	....	5	....
Feb. 15	4	27.5	....	30	....	27	....
Feb. 26	7	27.5	....	62	....	55	....
Mar. 13	3	33.1	....	29	....	24	....
Mar. 14	4	30.9	....	58	....	52	....
Mar. 15	5	34.7	....	20	....	13	....
Mar. 16	6	24.2	....	33	....	30	....
Mar. 17	7	27.5	....	31	....	24	....
Mar. 31	3	33.1	....	12	....	7	....
Apr. 1	4	29.8	....	25	....	22	....
Apr. 3	6	33.1	....	37	....	29	....
Apr. 4	7	34.7	....	75	....	65	....
Apr. 5	8	33.7	....	30	....	17	....
Apr. 6	5	30.9	....	11	....	4	....
Apr. 15	5	26.5	....	33	....	29	....
Apr. 17	7	26.5	....	28	....	21	....
Apr. 18	8	24.2	....	22	....	18	....
Apr. 19	4	25.5	....	37	....	34	....
Apr. 20	5	28.8	....	31	....	27	....
Apr. 21	6	35.3	....	44	....	36	....
Apr. 22	7	33.1	....	50	....	40	....
Apr. 24	4	29.8	....	38	....	35	....
Apr. 25	5	32.0	....	26	....	19	....
Apr. 26	6	32.0	....	30	....	22	....
Apr. 27	7	32.0	....	51	....	41	....
May 4	7	27.5	....	15	....	8	....
May 15	3	21.0	....	11	....	10	....
May 16	3	22.7	....	10	....	9	....
May 17	4	21.5	....	3	....	2	....
May 18	5	21.5	....	7	....	5	....
Do	5	21.5	....	8	....	6	....
Do	5	21.5	....	5	....	3	....
Do	5	21.5	....	5	....	3	....
Do	5	21.5	....	7	....	5	....
May 19	7	20.5	....	9	....	6	....
May 22	9	20.0	....	7	....	1	....
Do	10	20.0	....	29	....	21	....

TABLE V.—*Detailed study of various muscicides*—Continued.

ARSENITE SOLUTION, ONE THOUSANDTH NORMAL.

Date.	Age.	Temperature.	Mortality (crude).		Mortality (corrected).		Coefficient.
			Test.	Standard.	Test.	Standard.	
1916.	*Days.*	°*C.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
May 24	11	20.0		13		0	
May 25	4	21.0		1		0	
May 26	5	22.0		17		15	
May 27	6	23.2		18		15	
May 29	8	24.2		12		8	
May 31	10	23.2		11		3	
June 1	11	22.0		10		0	
June 2	4	22.0		6		5	
June 3	5	22.0		1		0	
June 5	7	21.5		9		6	
Average		27.3				21	1
ARSENITE SOLUTION, ONE HUNDREDTH NORMAL.							
May 26	5	22.0	66	17	64	15	
May 27	6	23.0	77	18	74	15	
May 31	10	23.0	56	9	48	1	
Average		22.7			62	10	6.2
ARSENITE SOLUTION, ONE THOUSANDTH NORMAL, IN MILK.							
Apr. 4	7	34.0	70	75	60	65	
Apr. 5	8	33.5	15	30	2	17	
Do	8	33.5	18	30	5	17	
Average		33.7			22	33	0.67
ALUMINUM CHLORIDE SOLUTION, 1 PER CENT.							
Mar. 13	3	33.1	13	29	8	24	
Mar. 14	4	30.9	35	58	29	52	
Mar. 15	5	34.2	19	20	12	13	
Mar. 16	6	24.2	25	33	22	26	
Average		30.6			18	29	0.62
SODIUM BENZOATE SOLUTION, 1 PER CENT.							
Mar. 13	3	31.1	51	29	46	24	
Mar. 14	4	30.9	22	58	16	52	
Mar. 15	5	34.2	9	20	2	13	
Mar. 16	6	24.2	8	33	5	30	
Average		30.1			17	29	0.59
SODIUM CHLORATE SOLUTION, 1 PER CENT.							
Mar. 17	7	27.5	2	31	0	24	
Mar. 31	3	36.5	1	12	0	7	
Apr. 1	4	29.8	23	25	20	22	
Apr. 3	6	31.1	20	37	15	32	
Average		31.2			9	21	0.43
SODIUM CITRATE SOLUTION, 1 PER CENT.							
Mar. 13	3	33.1	3	29	0	24	
Mar. 14	4	30.9	8	58	2	52	
Mar. 15	5	34.2	6	20	0	13	
Mar. 16	6	24.2	16	30	13	27	
Average		30.6			4	29	0.14

TABLE V.—*Detailed study of various muscicides*—Continued.

POTASSIUM DICHROMATE, ONE HUNDREDTH NORMAL.

Date.	Age.	Temperature.	Mortality (crude).		Mortality (corrected).		Coefficient.
			Test.	Standard.	Test.	Standard.	
1915.	*Days.*	*° C.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Nov. 22	3	30–35	14	25	9	20	....
Nov. 23	4	30–35	18	25	12	19	....
Nov. 26	7	30–35	41	56	31	46	....
Nov. 30	11	30–35	48	44	33	19	....
Average	....	....	....	....	21	26	0.81

FORMALDEHYDE SOLUTION, ONE-HALF PER CENT.

Date.	Age.	Temperature.	Mortality (crude). Test.	Mortality (crude). Standard.	Mortality (corrected). Test.	Mortality (corrected). Standard.	Coefficient.
1916.							
Feb. 25	6	32.0	84	39	76	31	....
Feb. 26	7	27.5	80	62	73	55	....
Do	7	27.5	79	62	72	55	....
Do	7	27.5	79	62	72	55	....
Do	7	27.5	93	62	86	55	....
Apr. 4	7	34.2	77	75	67	65	....
Apr. 7	6	32.0	47	38	39	30	....
Apr. 8	7	33.1	60	47	50	37	....
Apr. 17	7	26.5	64	28	57	21	....
Do	7	26.5	34	28	37	21	....
Do	7	26.5	38	28	31	21	....
Apr. 18	8	24.2	54	22	50	18	....
Do	8	24.2	47	22	43	18	....
Do	8	24.2	58	22	54	18	....
Apr. 19	4	25.5	92	37	89	34	....
Do	4	25.5	76	37	73	34	....
Do	4	25.5	97	37	94	34	....
Apr. 22	7	33.1	77	50	67	40	....
Apr. 24	4	29.8	71	38	68	35	....
Apr. 25	5	32.0	56	26	49	19	....
Apr. 26	6	32.0	81	30	73	22	....
Apr. 27	7	32.0	80	51	70	41	....
May 4	7	27.5	58	15	51	8	....
May 15	2	21.0	55	11	54	10	....
Do	2	21.0	57	11	56	10	....
May 16	3	22.7	30	10	29	9	....
Do	3	22.7	31	10	30	9	....
May 17	4	21.5	25	3	24	2	....
Do	4	21.5	60	3	59	1	....
May 19	6	25.5	27	9	22	4	....
May 22	9	20.0	36	7	30	1	....
Do	9	20.0	33	7	27	1	....
May 23	10	20.0	32	29	24	21	....
May 24	11	20.0	28	13	14	0	....
Do	11	20.0	31	13	17	0	....
May 25	4	21.0	36	1	35	0	....
May 26	5	22.0	48	17	46	15	....
May 29	8	24.2	47	12	43	8	....
Do	8	24.2	35	12	31	8	....
Average	....	26.6	....	....	51	22	2.32

FORMALDEHYDE SOLUTION, 1 PER CENT.

Date.	Age.	Temperature.	Mortality (crude). Test.	Mortality (crude). Standard.	Mortality (corrected). Test.	Mortality (corrected). Standard.	Coefficient.
Jan. 21	6	32.0	77	39	69	31	....
Feb. 4	2	30.9	59	10	54	5	....
Feb. 15	4	27.5	60	30	57	27	....
Do	4	27.5	68	30	65	27	....
Do	4	27.5	72	30	69	27	....
Do	4	27.5	76	30	73	27	....
Feb. 25	5	33.1	79	59	72	52	....
Average	....	29.4	....	....	66	28	2.36

FORMALDEHYDE SOLUTION, 4 PER CENT.

Date.	Age.	Temperature.	Mortality (crude). Test.	Mortality (crude). Standard.	Mortality (corrected). Test.	Mortality (corrected). Standard.	Coefficient.
Jan. 7	4	33.1	69	41	63	35	....
Jan. 10	7	32.0	44	29	34	20	....
Jan. 11	8	29.8	43	44	34	25	....
Jan. 12	9	33.1	72	44	57	29	....
Average	....	32.0	....	....	47	27	1.74

TABLE V.—*Detailed study of various muscicides*—Continued.

FORMALDEHYDE SOLUTION, 8 PER CENT.

Date.	Age.	Temperature.	Mortality (crude).		Mortality (corrected.)		Coefficient.
			Test.	Standard.	Test.	Standard.	
1916.	*Days.*	*°C.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Jan. 20	4	30.9	23	28	17	22	....
Average		30.9	....	....	17	22	0.77

FORMALDEHYDE SOLUTION, ONE-HALF PER CENT+MOLASSES, 10 PER CENT.

Apr. 7	6	32.0	52	38	44	30	....
Do	6	32.0	74	38	66	30	....
Do	6	32.0	35	38	27	30	....
Apr. 22	7	33.1	47	50	37	40	....
Apr. 24	4	29.8	50	38	47	35	....
Average	....	31.8	....	....	44	33	1.33

FORMALDEHYDE SOLUTION, ONE-HALF PER CENT+BROWN SUGAR, 10 PER CENT.

Apr. 8	7	33.1	79	47	69	37	....
Do	7	33.1	62	47	52	37	....
Do	7	33.1	52	47	42	37	....
Apr. 25	5	32.0	59	26	52	19	....
Apr. 26	6	32.0	64	30	56	22	....
Apr. 27	7	32.0	64	51	54	41	....
Average	....	32.6	....	....	54	32	1.69

PARAFORMALDEHYDE (DRY POWDER).

May 5	8	24.2	6	32	2	28	....
Do	8	24.2	10	32	6	28	....
Average	....	24.2	....	....	4	28	0.14

PARAFORMALDEHYDE (SATURATED SOLUTION).

May 5	8	24.2	30	32	26	28	....
June 5	7	21.5	23	9	20	6	....
Do	7	21.5	14	9	11	6	....
Do	7	21.5	19	9	16	6	....
Do	7	21.5	24	9	21	6	....
Average	....	22.0	....	....	19	10	1.90

SODIUM FLUORIDE SOLUTION, 1 PER CENT.

Mar. 17	7	27.5	65	31	58	24	....
Mar. 31	3	36.2	51	12	46	7	....
Apr. 1	4	29.8	89	25	86	22	....
Apr. 3	6	33.1	86	37	78	29	....
Average	....	31.7	....	....	67	21	3.19

POTASSIUM IODIDE SOLUTION, 1 PER CENT.

Mar. 17	7	27.5	2	31	0	24	....
Mar. 31	3	36.2	2	12	0	7	....
Apr. 1	4	29.8	55	25	52	22	....
Apr. 3	6	33.1	13	37	5	29	.....
Average	....	31.7	....	....	11	21	0.67

TABLE V.—*Detailed study of various muscicides*—Continued.

POTASSIUM NITRATE SOLUTION, 1 PER CENT.

Date.	Age.	Temperature.	Mortality (crude).		Mortality (corrected).		Coefficient.
			Test.	Standard.	Test.	Standard.	
1916.	*Days.*	°*C.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Mar. 17	7	27.5	20	31	13	24	....
Mar. 31	3	36.2	1	12	0	7	....
Apr. 1	4	29.8	9	25	6	22	....
Apr. 3	6	33.1	15	37	7	29	....
Average	....	31.7	....	....	7	21	0.33

QUASSIA SIRUP (RECOMMENDED BY UNITED STATES DISPENSATORY).

1915.							
Dec. 14	4	30.9	4	20	0	14	....
Dec. 15	5	30.9	15	27	8	20	....
Dec. 16	6	29.8	9	31	4	26	....
Average	....	30.5	....	....	4	20	0.20

SODIUM SALICYLATE SOLUTION, 1 PER CENT.

1916.							
Mar. 13	3	33.1	34	29	29	24	....
Mar. 14	4	30.9	48	58	42	52	....
Mar. 15	5	34.2	54	20	47	13	....
Mar. 16	6	24.2	62	33	59	30	....
Apr. 15	5	26.5	36	33	32	29	....
Apr. 19	4	25.5	45	37	42	34	....
Apr. 20	5	28.8	78	31	74	27	....
Do.	5	28.8	55	31	51	27	....
Do.	5	28.8	52	31	48	27	....
Do.	5	28.8	40	31	36	27	....
Apr. 21	6	35.3	43	44	35	36	....
Do.	6	35.3	29	44	21	36	....
Do.	6	35.3	31	44	23	36	....
Do.	6	35.3	43	44	35	36	....
Apr. 22	7	33.1	55	50	45	40	....
Apr. 24	4	29.8	74	38	71	35	....
Apr. 25	5	32.0	43	26	36	19	....
Apr. 26	6	32.0	56	30	48	22	....
Apr. 27	7	32.0	55	51	45	41	....
Average	..	31.0	....	....	43	31	1.39

SODIUM SALICYLATE SOLUTION, 1 PER CENT+BROWN SUGAR, 10 PER CENT.

Apr. 15	5	26.5	45	33	41	29	....
Apr. 25	5	32.0	66	26	59	19	....
Apr. 26	6	32.0	63	30	55	22	....
Apr. 27	7	32.0	44	51	34	41	....
May 15	2	21.0	51	11	50	10	....
Do.	2	21.0	74	11	73	10	....
May 17	4	21.5	7	3	6	2	....
Do.	4	21.5	13	3	12	2	....
May 19	6	20.5	46	9	43	6	....
May 22	9	20.0	51	7	45	1	....
Do.	9	20.0	29	7	23	1	....
May 23	10	20.0	28	29	20	21	....
Do.	10	20.0	52	29	44	23	....
May 24	11	20.0	12	13	0	0	....
May 25	4	21.0	28	1	27	0	....
Do.	4	21.0	14	1	13	0	....
May 26	5	22.0	25	17	23	15	....
May 27	5	22.0	15	18	13	16	....
May 29	8	24.2	28	12	24	8	....
Do.	8	24.2	28	12	24	8	....
Average	....	23.1	....	....	32	12	2.66

TABLE V.—*Detailed study of various muscicides*—Continued.

SODIUM SALICYLATE SOLUTION, 1 PER CENT+MOLASSES, 10 PER CENT.

Date.	Age.	Temperature.	Mortality (crude).		Mortality (corrected).		Coefficient.
			Test.	Standard.	Test.	Standard.	
1916.	*Days.*	° *C.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Apr. 15	5	26.5	25	33	21	29	....
Do.	5	26.5	12	33	8	29	....
Apr. 22	7	33.1	54	50	44	40	....
Apr. 24	4	29.8	54	38	51	35	....
Average	....	29.0	....	....	31	33	0.94

SPECIAL STUDY OF CERTAIN MUSCICIDES.

Temperature effect.—The study of these substances was carried out over the period from February 25, 1916, to June 1, 1916. Up to May 4, 1916, the building where the experiments were conducted was artificially heated to an average temperature of 30.6° C., but on that date the heat was shut off. Immediately following this the outside temperature dropped and for a period of three weeks the tests were carried out at a temperature which averaged 21.9° C. A marked difference in the effect of the poisons was noticed with the lowering of the temperature. Tests were made of the formaldehyde, sodium salicylate, and arsenic at the lower temperature. A comparison of the corrected average mortalities due to the standard arsenic and the two poisons at the two ranges of temperature is given in Table VI, computed from the original data of Table V.

TABLE VI.—*Temperature effect upon the relative efficiency of certain muscicides.*

Muscicide.	Average temperature.	Average mortality, corrected.	Decrease at lower temperature.
	° *C.*	*Per cent.*	*Per cent.*
Standard arsenite solution	30.6 21.9	25 6	76.0
0.5 per cent formaldehyde	28.5 21.7	63 34	46.0
1 per cent sodium salicylate + 10 per cent brown sugar	30.6 21.2	47 27	42.5

This rapid and disproportionate decrease in the effect of the arsenite standard has the result of raising the coefficient of the other substances at lower temperatures. This necessitates the arbitrary selection of a fixed temperature for any standard comparative work. It is preferred, in the present discussion, however, to state both the coefficient obtained and the actual temperature of the test, and to interpret the result in the light of the facts just given rather than to attempt to reduce the coefficients to a standard temperature.

Most effective concentration of formaldehyde.—With the wide variation in the concentrations of formaldehyde recommended for use, the need of determining the most effective strength was at once apparent. A series of tests were made, using concentrations varying from 0.062 per cent to 1 per cent. Table VII shows the results of this special series. For comparison data from Table V with 4 and 8 per cent formaldehyde are also included.

TABLE VII.—*Effect of concentration of formaldehyde on its killing power.*

Date.	Age.	Temperature.	Concentration.	Mortality (crude).		Mortality (corrected).		Coefficient.
				Test.	Standard.	Test.	Standard.	
1916.	*Days.*	° C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Feb. 25	6	32.0	0.062	16	39	8	31	0.26
Do	6	32.0	.125	24	39	16	31	.52
Do	6	32.0	.25	56	39	48	31	1.55
Do	6	32.0	.5	84	39	76	31	2.45
Do	6	32.0	1.0	77	39	69	31	2.22
			4.0	(1)	(1)	47	27	1.74
			8.0	(1)	(1)	17	22	.77

[1] See Table V.

From this single set of results it appears that the most effective range of concentration is from 0.5 to 1 per cent. The slight superiority of the lower concentration is of little significance, and it will be seen later that it disappears in a more extensive series. Below this concentration insufficient substance is taken to kill and in more concentrated solutions the odor is apparently strong enough to be repellant.

Attractiveness.—It is a matter of some interest in the study of a muscicide whether it is specifically attractive or repellant to flies. It may be assumed that a fly will drink, under stress of necessity, from a solution that is normally repellant. Under normal conditions, however, there are usually alternative drinking places, and a successful poison must be at least nonrepellant and preferably attractive. Formaldehyde in a concentration of from 0.5 to 1 per cent has a decided odor, which, however, does not appear to be repellant to the flies. On the contrary, the following experiment furnishes some evidence that this odor is actually attractive. Four cages, each containing 100 flies 7 days old, were arranged in the usual manner. In each cage there was placed a dish of 0.5 per cent formaldehyde. In cage No. 2 there was placed also a dish of tap water, in cage No. 3 two dishes of tap water, and in cage No. 4 three dishes of tap water. The temperature during the test was 29.8° C.

The experiment was based upon the following reasoning: If the formaldehyde is neither attractive nor repellant, then the flies will drink from the various dishes at random, their selection in the long run being governed wholly by the law of chance. Of the total opportunities for drinking, all are poisonous in cage No. 1, one-half in cage No. 2, one-third in cage No. 3, and one-fourth in cage No. 4. If the law of chance is alone at work, therefore, the relative numbers killed should be in the same proportion, namely, 1, 0.5, 0.33, 0.25, respectively. Table VIII gives the results obtained and for comparison, the computed results based upon the reasoning employed above. In each case there is actually a higher death rate than that computed.

Table VIII.—*Test of attractiveness of 0.5 (per cent) formaldehyde solution.*

Cage.	Mortality (per cent).		
	Crude.	Corrected.	Theoretical.
1	58	51	51
2	39	32	26
3	26	19	17
4	22	15	13

This evidence is, of course, quite limited, but it is consistent, and points toward the fact that there is at least no repellant action and probably a slight attractiveness in the odor of formaldehyde.

The action of formaldehyde of this strength is very rapid. Upon a number of occasions it was actually noted that approximately three minutes elapsed between the drinking of the solution and death. The considerations of the experiment just cited are therefore not complicated by the possibility of the flies drinking, on the average, more than once within the four-hour period.

A similar test of the attractiveness of a 1 per cent sodium salicylate solution was also made. Five-day old flies were used. The room temperature during the test was 32.5° C. The results obtained, and for comparison, the computed results based upon the law of chance, are given in Table IX.

Table IX.—*Test of attractiveness of 1 per cent sodium salicylate solution.*

Cage.	Mortality (per cent).		
	Crude.	Corrected.	Theoretical.
1	41	34	34
2	11	4	17
3	14	7	11
4	8	1	9

The evidence in this case is decidedly negative. It is safe to say that a 1 per cent solution of sodium salicylate is somewhat repellant and that the flies prefer water. The increase in the killing coefficient brought about by the addition of brown sugar is no doubt due to the neutralizing of this repellant action. Reference to Table V will show this effect. The coefficient without sugar was 1.39 and with 10 per cent of sugar added 1.67, both at 31° C.

Comparison with commercial arsenic papers.—In order to compare the formaldehyde and sodium salicylate solutions with the commercial fly poisons on the market, samples of four different poison fly papers were prepared according to the directions which accompanied them. These solutions were tested by the standard procedure with the results shown in Table X.

TABLE X.—*Test on commercial arsenic fly poison prepared as directed on the label.*

Date.	Sample No.	Temperature.	Age of flies.	Mortality (crude).	Mortality (corrected).
1916.		°*C.*	*Days.*	*Per cent.*	*Per cent.*
June 1	1	22.0	11	32	18
Do	2	22.0	11	13	0
June 2	3	22.0	4	65	64
Do	4	22.0	4	47	46

The percentage of flies killed by the commercial papers is no greater, at least, than the percentage killed under similar conditions by either the formaldehyde or the sodium salicylate, and with some papers it is distinctly less.

SUMMARY OF EXPERIMENTAL RESULTS.

Two solutions, formaldehyde in 0.5 to 1 per cent, and sodium salicylate in 1 per cent concentration, have been found to be at least as efficacious as the customary arsenic preparations. The efficiencies are from two to three times that of the standard arsenite solution at summer temperatures and do not suffer the marked lowering at lower temperatures so strikingly shown in the cases of the arsenite. Both are safe for domestic use. The formaldehyde is slightly more efficient as a muscicide. The salicylate is more readily prepared and more permanent, being nonvolatile, and does not involve the keeping on hand of a rather powerful and objectionable substance.

PRACTICAL INTERPRETATION OF CORRECTED MORTALITY FIGURES.

NUMERICAL.

While the mortality figures obtained by the use of the various poisons are important in that they give a means of ready comparison, they do not show directly the percentage of flies which would be killed daily under conditions similar to those of the test. An attempt has therefore been made to determine this relation. All of these tests were started between 9.30 and 12 a. m. and finished between 1.30 and 4 p. m. There is no evidence to show that this period was typical of the 24 hours of the day or that similar results would be obtained at an earlier or a later period of the day.

Cages containing one hundred 4 to 5 day old flies each were exposed to a solution containing 1 per cent sodium salicylate plus 10 per cent brown sugar for 4-hour periods beginning at 4 a. m. and ending at 8 p. m. The test was conducted on May 9, 1916, at a temperature of 23.2° C. Up to the time the solutions were exposed the flies had free access to food and water. The counts were made during the following day. The results of the test are as follows:

TABLE XI.—*Mortality rate as a function of time of day.*

Time of day exposed.	Mortality (crude).	Mortality (corrected).	Daily mortality.
	Per cent.	*Per cent.*	*Per cent.*
4 a. m. to 8 a. m.	34	33	45.8
8 a. m. to 12 noon	24	23	32.0
12 noon to 4 p. m.	8	7	9.7
4 p. m. to 8 p. m.	10	9	12.5

Averaging the corrected mortalities from 8 a. m. to 4 p. m., a mortality of 15 per cent is obtained. As this is the time during which all of the tests of the various poisons were made, it is seen that had the tests been made between 4 and 8 a. m. the percentage of mortality would have been increased approximately two-fold. If the determinations had been made between 4 and 8 p. m. only one-half as many would have died. Assuming, as seems probable, that the feeding between 8 p. m. and 4 a. m. is negligible, the average mortality rate for the 24 hours is 12 per cent per 4 hours, a value in sufficient agreement with the average value of 15 per cent obtained above to justify the assumption that the mortality rates here obtained are approximately correct mean values over a 24-hour period. The consecutive application of a mortality rate of 12 per cent per 4 hours to a fixed population gives a total rate per 24 hours of 53 per cent. For convenience the relation between certain 4-hour and the corresponding 24-hour mortality rates is given:

Equivalent mortality rates.

4 hours.	24 hours.
Per cent.	*Per cent.*
10	47
20	74
30	88
40	95
50	99

A further consideration in applying these results is the relative proportion of the total floor areas available and the size of the poison-containing dish. It will readily be seen that the dish in these experiments occupied a relatively large proportion of the available floor and wall space. In a larger room the relative size of the dish would be smaller in proportion, and the chance of a fly alighting near it by accident correspondingly reduced.

This factor is partially offset, on the other hand, by the factor of attractiveness.

ATTRACTIVE AGENTS.

On comparing the results of the killing effect of the simple poison solutions and those also containing substances which are known to attract flies, such as sugar, molasses, or milk, it is frequently found that the coefficient is lower when the "attractive" agent is present. On the face of this evidence it would seem that no benefit was derived by the addition of such substances. Under the conditions of the test the surface of the poison solution is a much greater percentage of the total area of the floor space than it would be in practical use. Also the flies are more congested than would generally be the case. Whether the solution has any attractiveness or not, all the flies will no doubt find it in the course of four hours. The decrease in killing coefficient in these cases, therefore, means a decrease in actual toxicity of the solution caused by the addition of the organic matter. In connection with what has been said concerning the size of the room it is to be noted that increased attractiveness would be more noticeable in a larger room and might offset to a large extent the factor of decreased relative size of the dish.

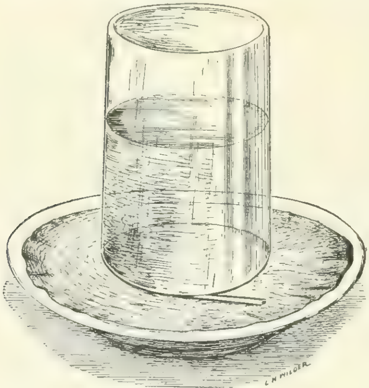

FIG. 2.—Container for fly poison.

USE OF MUSCICIDES.

PREPARATION OF SOLUTIONS.

For use in the household a formaldehyde solution of approximately the correct strength may be made by adding 3 teaspoonfuls of the concentrated formaldehyde solution, commercially known as formalin, to a pint of water. Similarly, the proper concentration of sodium salicylate may be obtained by dissolving 3 teaspoonfuls of the pure chemical (a powder) to a pint of water.

As commercial formaldehyde solution, on account of its strong odor, is not easily handled, it is suggested that druggists and drug houses prepare a 1 per cent solution for this purpose and retail it at a relatively low cost. It is also suggested that the sodium salicylate might be sold in the form of a concentrated solution with directions for diluting to 1 per cent, or possibly sheets of absorbent paper impregnated with a known amount of the dry substance, similar to the arsenic papers now on the market, might be sold.

CONTAINERS FOR SOLUTIONS.

A container such as is shown in Figure II has been found convenient for automatically keeping the solution always available for flies to drink. An ordinary, thin-walled drinking glass is filled or partially filled with the solution. A saucer, or small plate in which is placed a piece of white blotting paper cut the size of the dish, is put bottom up over the glass. The whole is then quickly inverted, a match placed under the edge of the glass, and the container is ready for use. As the solution dries out of the saucer the liquid seal at the edge of the glass is broken and more liquid flows into the lower receptacle. Thus the paper is always kept moist.

STICKY FLY PAPERS.

The experimental work done on sticky fly preparations had for its object the study of the compounding of a sticky preparation having attractive properties, and the comparison of the sticky and poison preparations as to their efficiency as fly killers.

COMPOUNDING AND TESTING OF STICKY FLY PREPARATIONS.

In general, sticky preparations have for their basis a solution of rosin in an oil. A number of oils were tried, mixed with both white and grade E rosin. The selection of oils was made with partial reference to their possible attractiveness. The cheaper impure rosin was eliminated after a few tests, for it was found that the impurities contained in it prohibited the obtaining of a homogeneous mixture of the rosin and oil, and some evidence was also obtained that the strong odor of the crude rosin had more of a repellant action than that which originated from the purer product.

The sticky substance was heated in boiling water till it became fluid. It was then spread over 6-inch squares of unsized manila wrapping paper. These papers were made in triplicate, and were placed on the floor of the fly breeding room where the flies were very numerous, and were left exposed from 2 p. m. to 9.30 a. m. of the following day. They were then removed and a count made of the catch.

Table XII gives the preparations used and the results obtained.

TABLE XII.—*Results of tests on sticky preparations.*

Date.	Preparation No.	Oil.		White rosin parts by weight.	Flies caught (average).
		Kind.	Parts by weight.		
1915.					
Dec. 9	1	Castor	2	5	35
9	2	Olive	2	5	1
9	3	Lard	2	5	8
9	4	Sperm	2	5	1
9	5	Sturgeon	2	5	1
9	6	Cod liver	2	5	1
9	7	“Fish”	2	5	7
9	Commercial				25
10	8	Castor	3	10	3
10	9	Olive	3	10	1
10	10	Lard	3	10	0
10	11	Sperm	3	10	3
10	12	Sturgeon	3	10	5
10	13	Cod liver	3	10	5
10	14	“Fish”	3	10	4
10	Commercial				32
11	15	Castor	4½	10	38
11	16	do	5	10	61
11	Commercial				55

It is seen that the castor-oil preparations are far superior to the others. Preparation No. 16, composed of 1 part by weight of castor oil and 2 parts of white rosin, is the most satisfactory. This mixture gives as good results as the common sticky preparation on the market.

These experiments have not disclosed, therefore, any advantage in the use of supposedly attractive oils.

IMPROVEMENTS IN HANDLING STICKY PREPARATIONS.

It is believed, however, that certain improvements are possible in the manner of handling these preparations with a view to making them less objectionable both as to appearance and as to danger of accidental contact with clothing, furniture, and books. The preparation No. 16 can be diluted with a small amount of ether and put up in liquid form in a bottle. This liquid is readily applied to a plate or cup and can be removed in boiling water with washing powder. While this particular solvent is not free from objections, its price being one, it is believed that the manufacture of a suitable liquid preparation, for such use as is indicated, would lead to the increased use of sticky preparations.

A semi-inclosed container, to the inside of which the preparation could be applied, would also be a distinct improvement from an esthetic viewpoint and would permit the use of attractive agents to increase further the efficiency of the device.

COMPARISON OF EFFICIENCIES OF STICKY PAPERS AND MUSCICIDES.

Tests were conducted to determine the relative efficiency of the sticky paper and the muscicides. Squares of commercial sticky paper and dishes of poison solution having the same area were exposed in separate experimental cages to flies under standard conditions. The following results were obtained:

Temperature, ° C.	22.7
Mortality, per cent (corrected):	
0.5 per cent formaldehyde	34.0
1 per cent sodium salicylate+10 per cent brown sugar	33.0
Sticky paper	14.0

About two and one-half times as many flies were killed by the muscicides as by the sticky papers under the conditions of the experiment.

SUMMARY AND CONCLUSIONS.

The use of muscicides or fly poison preparations has many distinct advantages over other methods of combating the fly nuisance within the household. A serious drawback to this method has heretofore been the extremely poisonous character of the substances available and the consequent danger, especially to children, attending their use.

A somewhat comprehensive survey has been made of other chemical substances having possible muscicidal properties with a view to substituting them for the arsenic preparations now commonly employed.

This study has necessitated the development of an experimental technique for the determination of relative muscicidal efficiencies of various preparations. The procedures developed permit the determination of a relative coefficient, one thousandth normal sodium arsenite, serving as a standard basis of comparison.

Of the substances frequently recommended, potassium dichromate and quassia sirup have been found to be of little value. Formaldehyde, on the other hand, when properly employed has been found to be much more efficient than the standard arsenite solution. The studies have indicated the most efficient strength of the formaldehyde solution to be from 0.5 to 1 per cent, which is equivalent to 1.25 to 2.5 per cent of the 40 per cent solution sold as formalin.

A muscicide of almost equal efficiency and of distinctly superior qualities in many ways has been found in the substance sodium salicylate, a 1 per cent aqueous solution of which is recommended.

At midsummer temperatures the efficiency of either of these preparations is slightly greater than that of solutions prepared from commercial poison papers. Directions for preparing these solutions in the household and recommendations for their commercial preparation and sale are made.

Sticky fly papers have been investigated with special reference to a possible improvement in their quality by giving them added attractiveness to flies and the question of a more suitable manner of use has been discussed. No improvements have been discovered in the direction of efficiency. It has been found possible to prepare a liquid compound which can be used in more convenient and less unsightly containers than those at present employed, and the preparation and marketing of some similar compound upon the part of those engaged in the manufacture of the present papers is recommended. The relative efficiencies of the sticky preparations and the muscicides have been studied and, on the basis of equal areas exposed, the latter have been found to be several times as efficient under the experimental conditions.

ACKNOWLEDGMENTS.

The sticky compounds used in these studies were prepared by Mr. Elias Elvove, technical assistant, Hygienic Laboratory, to whom the authors' thanks are expressed and to whom they are indebted also for an extensive survey of the literature bearing upon this question. In the experimental portion of the work valuable assistance has been rendered by Mr. A. A. Moore, laboratory attendant, which is also gratefully acknowledged.

HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891; and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe. (*B. typhi murium* Danyz applied to the destruction of rats.) By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

†No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

†No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

*No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis*, by Ch. Wardell Stiles.

*No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

*No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

*No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The international code of zoological nomenclature as applied to medicine. By Ch. Wardell Stiles.

*No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

*No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

*No. 27.—The limitations of formaldehyde gas as a disinfectant, with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

†No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

†No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

†No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

*No. 33.—Studies in experimental alcoholism. By Reid Hunt.

†No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

†No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

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†No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

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TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY—BULLETIN No. 109

DECEMBER, 1916

PITUITARY STANDARDIZATION. THE RELATIVE VALUE OF INFUNDIBULAR EXTRACTS MADE FROM DIFFERENT SPECIES OF MAMMALS AND A COMPARISON OF THEIR PHYSIOLOGICAL ACTIVITY WITH THAT OF CERTAIN COMMERCIAL PREPARATIONS.

By GEORGE B. ROTH

PHARMACOLOGICAL STUDIES WITH COCAINE AND NOVOCAINE. A COMPARATIVE INVESTIGATION OF THESE SUBSTANCES IN INTACT ANIMALS AND ON ISOLATED ORGANS.

By GEORGE B. ROTH

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PITUITARY STANDARDIZATION. THE RELATIVE VALUE OF INFUNDIBULAR EXTRACTS MADE FROM DIFFERENT SPECIES OF MAMMALS AND A COMPARISON OF THEIR PHYSIOLOGICAL ACTIVITY WITH THAT OF CERTAIN COMMERCIAL PREPARATIONS.[1]

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INTRODUCTION.

The search for a definite chemical principle in infundibular extracts[2] which will simulate the action of these extracts (made from the posterior lobe of the pituitary body) has been vigorously pursued for many years. Up to the present time chemists and physiologists are undecided as to whether the activity of such extracts is due to one or several principles. The interest of physicians in infundibular extracts is to-day largely centered upon their value in therapeutics. The investigation of the physiological activity of commercial infundibular preparations is very necessary before a proper therapeutic value can be placed upon them. It is also highly important to know whether or not the commercial preparations possess a uniform activity if advances are to be made in therapeutics.

The present methods for estimating the activity of infundibular extracts are for the most part physiological, the chemical tests which have been elaborated being qualitative in nature. Inasmuch as the amount of active substances in infundibular extracts can now be determined by physiological tests, it is a matter of concern to the manufacturer and of interest to the physiologist to have an estimate of the active substances in extracts made in various ways.

Commercial infundibular extracts have been shown by Roth (1914) to be variable in their activity, but no positive reason could be given for the wide difference in their variability. Such variability might be caused by a number of factors, among them being the method of preparation and the animal source of the raw material.

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[1] Manuscript submitted for publication May 29, 1916.

[2] Pituitary extracts made from the posterior lobe of the pituitary body have properly been named infundibular extracts by certain writers and will be referred to in this bulletin as such when the extracts from the posterior lobe of the pituitary body are spoken of. The term "neurohypophysin" may appropriately be used to designate posterior lobe extracts, while "glandulohypophysin" could be applied to anterior lobe extracts.

In the present investigation a study of some of the probable causes of such variability was made, together with a study of the relative activity of infundibular extracts made experimentally in the laboratory and of those obtained in the open market.

PART 1.

RECENT LITERATURE PERTAINING TO PITUITARY BODY.

Inasmuch as the recent literature pertaining to the pituitary body bears directly upon many points raised in this paper, it would appear desirable to present a brief résumé of some of the studies which are most pertinent to the subject. A partial survey of the early literature will be found in Hygienic Laboratory Bulletin No. 100. The literature to be reviewed in the present bulletin will cover the years elapsing since the appearance of the above-mentioned bulletin, except in a few instances where it is necessary again to call attention to certain work for the sake of clearness in the present paper. Excellent reviews will be found in compiled form in Biedl's Innere Sekretion, 1913, and in Cushing's exhaustive work entitled "The Pituitary Body and Its Disorders."

LITERATURE OF A CHEMICAL NATURE.

The physiological activity of infundibular extracts is generally thought to be due to some constituent other than those substances which have already been isolated from it, namely, calcium, phosphorus, arsenic, bromine, choline, and guanin. Unidentified substances have been described which have been called active principles, but upon investigation it was found that they simulate the activity of infundibular extracts only in part. It was at one time considered by some that the active substance was β-iminazolylethylamine, but this has been shown to be incorrect.

From the evidence thus far produced it appears that the activity of infundibular extracts is due to several constituents rather than to any one substance. The work of Schäfer and Vincent (1899) showed that the active substances were very stable, as they found that infundibular extracts could be boiled for some time without destroying their activity. They were able to separate the active fraction into two parts, which had opposite effects on the blood pressure of dogs; the pressor principle being soluble in water, while the depressor principle was insoluble in water, alcohol, or ether. The depressor principle was not identified, but it was regarded as distinctly unlike choline.

Baudouin (1913) dried the posterior lobe material in vacuo at a low temperature, defatted the dried material, and then made an acetic acid extract. After drying the acetic acid extract it was treated with hot alcohol, which on cooling precipitated a white substance which he considered to be the active principle. Baudouin was not able to extract as much of the active material from the fresh posterior lobe as from the dried gland. He found, moreover, that the precipitate which was formed upon the addition of acetic acid was practically inactive.

Heidelberg, Pittenger, and Vanderkleed (1914) prepared a metallic compound of the whole pituitary body in the following manner: A watery extract was first made, which was then deprived of practically all proteid substances. This purified extract was then treated with salts of aluminium or other suitable metals, after which it was neutralized, thus precipitating the active principles as a metallic compound. This product was soluble in water and exhibited all of the physiological properties of infundibular extracts.

Bouin and Ancel (1914) claim to have isolated the active substance by means of phosphotungstic acid. They fixed the fresh posterior lobe material in equal parts of absolute alcohol and ether for a period of about a week, then dried the material, powdered it and allowed it to macerate in water for several days. The aqueous filtrate was then neutralized with ammoniacal lead subacetate. The filtrate remaining after the neutralization with ammoniacal lead subacetate was acidulated with sulphuric acid and precipitated with phosphotungstic acid. This precipitate was washed with distilled water and .then treated with freshly prepared lead hydrate. This was then boiled for several minutes repeatedly to remove the active substance from the large number of impurities. After filtration the filtrate was finally precipitated by means of silver nitrate, the precipitate collected and washed and again rendered ammoniacal with ammonia water and filtered. The filtrate was then evaporated, leaving a crystalline material, which is a combination of silver with the active infundibular material. Ancel and Bouin (1914) carried the process further and, instead of forming a silver compound, obtained the pure base by extracting the mixture of lead hydrate and active precipitate by means of methyl alcohol. This extract was then treated with ether and crystallized in the cold.

Crawford and Ostenberg (1914) sought to isolate a pure pressor compound from the pituitary body. They extracted finely minced fresh beef glands with 0.1 per cent acetic acid by expression through cheesecloth, and coagulated the proteins by heating on a water bath and evaporating the filtrate in vacuo. This left a brownish yellow, gummy mass, which caused a marked rise in blood pressure when injected intravenously into dogs. On treatment with hot methyl alcohol all the color and activity were carried over in the alcohol. This solution could be precipitated by concentrated sulphuric acid. The precipitate was soluble in water and produced a marked rise in the blood pressure. They were unable to precipitate an active base by means of alkalies. By dialysis through heavy parchment paper much of the color of pituitary extracts could be removed, leaving in the dialyser a very active substance. If animal membranes were used in the dialysis the pressor principles were completely removed and could not be recovered. When the dialysate through the parchment paper was collected in fractions they found that the last few fractions were without activity, whereas the fluid in the dialyser was very active. It was suggested that the mother substance of the dialysable pressor principles is nondialysable, the depressor principle passing quickly into the dialysate.

Guggenheim (1914) found that in the commercial preparation "Pituglandol," in addition to proteinogenic amines such as occur in other organ extracts, there is a specific principle which has a characteristic action on the blood pressure and respiration and which, in contrast with certain other organic extracts, causes an increase in the tone of the uterus of the rat. The specific substance is inactivated by alkalies and can be absorbed to a marked degree by such substances as lead sulphide or talc. The specific substance somewhat resembled pilocarpine in its behavior toward alkalies. It is unlike β-iminazolylethylamine. both as regards its reaction toward alkalies and its effect on the uterus of the rat. He regards the active principle in Pituglandol as an ethereal combination of an alkanolamin with an acyl residue.

Aldrich (1915) prepared an aqueous extract from the desiccated. defatted posterior lobe and from it was able to separate what seemed to be histidine, or a compound of histidine which was in a more or less free state or in some combination other than with protein. No experiments were made to determine whether his compound was physiologically active nor to ascertain whether it behaved in the organism like infundibular extract made in the usual way.

Watanabe and Crawford (1916) were able to obtain color reactions from certain infundibular extracts which were similar to those given by suprarenal extracts. The physiological effects of such solutions, however, differed from those produced by suprarenal extracts. They explained this phenomenon by supposing that there existed in the solution an admixture of substances which mask the effect of the epinephrine. Their experiments, therefore, suggest the presence of epinephrine, or an epinephrine-like compound, in infundibular extracts.

LITERATURE OF A PHYSIOLOGICAL NATURE.

The early work of Oliver and Schäfer (1895) and of Howell (1898) on the physiological action of the pituitary body brought this structure into prominence as an organ worthy of further investigation by physiologists. They showed not only that extracts made from the pituitary body of mammals were physiologically active but that the activity resided mainly in the infundibular lobe. Herring (1908) confirmed these findings on birds, and further found that extracts of the pituitary body of the skate, which possesses only a rudimentary posterior lobe, were physiologically inactive.

Lewis, Miller, and Matthews (1911), working with the ox pituitary body, found that the more sharply the intermediary portion of the pituitary body was removed and extracted, the greater would be the rise in the blood pressure which resulted from the intravenous injection of such extracts; also that if a small amount of pars intermedia were used the rise in the blood pressure would be correspondingly less. Extracts of the pars intermedia were relatively more active than extracts made from the posterior lobe. They also obtained a depressor effect, followed by a pressor effect, from extracts made from the anterior lobe.

Herring (1914) was able to obtain an active extract from both the pars intermedia and the pars nervosa of the ox pituitary body, which would stimulate the isolated virgin uterus of the rat. The pars nervosa was from two to five times more active than the pars intermedia. In extracts of 0.5 per cent and under of dried material the pars intermedia had no specific effect on the blood pressure, kidney volume, or urinary secretion. On the other hand, extracts of the pars nervosa of a strength of 0.005 per cent of the dried material produced a prolonged rise in pressure when injected intravenously in two to three cubic centimeter doses, also an increase in kidney volume and of urine.

Fenger (1915) extracted the active substances from the posterior pituitary bodies of hogs and cattle by finely mincing the material and then extracting with slightly acidulated salt solution by boiling. The extract was then filtered, sterilized, and tested on the isolated uterus of the guinea pig. He found that the physiological activity, when determined in this way, was practically the same for hogs as for cattle, and that there was no distinct seasonal variation in the activity or chemical composition of the posterior lobe of the pituitary body of cattle.

Shamoff (1916) observed that certain commercial and laboratory preparations of infundibular extracts would stimulate and others inhibit the rhythmical movements of the isolated intestine of the rabbit. An extract, made from posterior-lobe material which had been dried in vacuo, was especially capable of producing intestinal inhibition, thus stimulating the action of epinephrine. He found that the substance was not constant in the extractives prepared in the usual ways, and, moreover, that it may be inconstant in the fresh glands from which these substances are prepared. It was suggested that its presence may depend upon the method of preparation. An effort was made to determine whether, in correspondence with their action on the intestinal segment, differences might arise as regards their blood pressure and diuretic properties, but there was nothing conclusive about such responses.

Hoskins (1916), using the intact intestine of the rabbit, confirmed the findings of Shamoff (1916). Hoskins obtained intestinal depression with every preparation of "Pituitrin" which he examined. He also obtained a similar response from saline extracts of old preparations of desiccated material.

LITERATURE OF A CLINICAL NATURE.

The clinical studies made to determine the therapeutic value of pituitary extracts are quite numerous. The majority of these studies were for the purpose of ascertaining the therapeutic value of infundibular extracts when used as uterine stimulants in labor or as a hemostatic agent in preventing postpartum hemorrhage.

Special attention should, perhaps, be directed to the literature bearing upon the use of infundibular extracts during labor, since many of the results obtained were injurious. Rupture of the uterus following the use of infundibular extract is a complication which has been frequently reported. The more recent literature contains many references to such an untoward result. Mention should be made, in this connection, of the fact that infundibular extract is administered subcutaneously in these cases, thereby securing rapid absorption. It is not unlikely that the dose has been excessive in a certain number of the cases. It would appear desirable that more accurate studies should be made to ascertain the dose to be employed, especially when given in uterine inertia.

Espeut (1913) reported rupture of the uterus, in a case of delayed parturition, after the injection of two doses of "pituglandol," administered one and one-half hours apart.

Grumann (1913) administered four injections of "pituitrin" at intervals of two hours to hasten labor, with the result that in a half hour after the last injection the contractions were so excessive that they kept up, practically continuously, for about two hours. The intense contractions destroyed the tissues to such an extent that a fistula was formed between the rectum and cervix. The child was finally delivered by other means and died on the seventh day from sepsis.

Harrison (1914) cautions against the use of more than a second dose of infundibular extract in delayed labor. He found that the effect came on in about six minutes after its administration, and lasted from 30 to 60 minutes. The second dose is advised after one hour. In certain cases considerable damage was done to the soft parts and to the child.

Quigley (1915) disagrees with those who contend that infundibular extracts are free from danger. He has found that one-half cubic centimeter of a solution of which 1 cubic centimeter represents 0.2 gram of dried substance was sufficient in every case. He has never used larger doses than 1 cubic centimeter, and never repeats the dose more than once. In his experience, the greatest danger which confronts those who employ infundibular extract in labor is rupture of the uterus. He reports a case in which the uterus ruptured after an initial dose of 4 cubic centimeters; and two other cases in which the same result followed the use of an initial dose of 1 cubic centimeter of infundibular extract.

Zueblin (1914) employed "pituitrin," in 1 cubic centimeter doses subcutaneously, in the treatment of acute dilatation of the heart and observed a beneficial effect following its administration.

Konikow (1915) considers infundibular extract as a powerful hemostatic agent in pulmonary hemorrhages, when given subcutaneously in doses representing 0.2 gram of the dried material.

Kahn and Gordon (1915), following the suggestion of Citelli, used infundibular extract, for the control of hemorrhage following nose and throat operations, with good results. They administered "pituitrin," subcutaneously in 12 to 15 minim doses, about 15 minutes before the anesthetic was given. They discovered that the coagulation time of the blood was materially reduced, and that the blood pressure effect was variable; however, in over half of the cases the blood pressure was raised.

Duffy (1915) summarizes much of the literature dealing with the use of infundibular extracts in intestinal stasis, and gives a résumé of his experience with it in post-operative intestinal stasis. He gave "pituitrin" in 1 c. c. doses, 6, 12, and 18 hours after laparotomies, with gratifying results. He concluded that infundibular extract is an important aid in post-operative ileus, and that it should be tried in all cases where purgatives are not retained by mouth. The effect on peristalsis in cases of tympanites seemed to be more marked than in cases with no intestinal distension.

Infundibular extract is also used to some extent in the treatment of shock, asthma, hay fever, and in urinary retention; also as a diuretic and as a galactogogue. The results obtained in these conditions are less convincing than in those previously mentioned.

PART 2.

SCOPE OF PRESENT INVESTIGATION.

The purpose of the present investigation was to ascertain the relative value of infundibular extracts made from various species of mammals, as well as to compare their physiological activity with that of certain commercial preparations. It was previously reported by the writer that commercial preparations of infundibular extracts varied greatly in activity. At that time it was suggested that a probable reason for their variability might be found in the animal source from which infundibular extracts were made, the material being obtained either from cattle, sheep, or horses. It was therefore deemed advisable to investigate, not only the yield of active constituents in the pituitary body of cattle, sheep, and horses, but in other mammals as well, in order to find out whether the present sources of the raw material are well chosen. The study was made to include cattle, sheep, horses, hogs, cats, dogs, and rabbits.

Inasmuch as it was shown elsewhere that the activity of commercial infundibular extracts, when measured by the isolated uterus method, was not strictly proportionate to the effect on the blood pressure, the activity was measured both by the isolated uterus and the blood pressure methods.

METHODS EMPLOYED.

Blood-pressure method.—In the blood-pressure method the effect of infundibular extract on the circulatory system is taken as an index of its activity when it is injected into a vein of an anesthetized dog. By comparison with a satisfactory standard, the amount of active pressor principle in infundibular extracts can be determined quantitatively. The actual method of procedure is essentially that used in the standardization of epinephrine and allied preparations. In brief, the method here employed was as follows: A sound and healthy dog was deeply anesthetized with chlorbutanol. This was dissolved in a small amount of alcohol and given by stomach, in an amount equivalent to about 0.4 gm. per kilo of animal weight, about one-half hour before the experiment. After complete anesthesia, the animal was tied securely and the skin and superficial neck tissues were dissected back from either side of the trachea, the trachea exposed and the carotid sheaths opened. The vagi on both sides were then freed from neighboring structures and cut. One carotid artery was then separated from the surrounding tissue, a cannula being inserted into it and connected to a mercury manometer for recording the blood-pressure effects on a smoked or smooth paper. A tracheal cannula was then introduced and connected with a respiration apparatus, in order to supply the animal with a constant amount of air. The femoral vein was then dissected from the surrounding structures and a cannula inserted into it. The animal was kept warm by means of an electric warming pad and an operating tank filled with water, which was kept at about the body temperature. Sufficient curarine was then given by the femoral vein to prevent spontaneous respiration. The preparations to be examined were then injected into the circulation through the cannula in the femoral vein and the effect on the blood pressure noted. Comparison was made with a standard. The amount of the unknown, required to raise the blood pressure to the same degree as it was raised by the standard solution, was ascertained by repeated trials, using unlike amounts of liquid. By the use of small amounts of infundibular extract, amounts which are much below that required to produce a maximum rise in pressure, the activity of these extracts can be determined with considerable accuracy.[1] The reaction at the close of an experiment is not as trustworthy as at the beginning, as the pressor effect is usually less marked when the blood contains a large amount of infundibular extract.

In certain respects the effect of infundibular extract and of epinephrine on the blood pressure is alike, and as the blood pressure effect of epinephrine is very fleeting, as compared with the effect produced by infundibular extract, it was thought that epinephrine, which is obtainable in a pure state and consequently less subject to variation, might be used as a standard. After repeated trials, it was found that epinephrine could not be used as successfully as a preparation of infundibular extract. In general, it was found that 1 c. c. of a 1 to 200,000 dilution of epinephrine chloride was equal in activity to 2 mgs. of fresh cattle infundibular material.[2] To show that the reaction to epinephrine and the reaction to infundibular extract do not vary in the same way the following will be cited. A small dog, anesthetized with chlorbutanol in the usual way, was given an intravenous injection of 1 c. c. of 1:200,000 epinephrine, causing a rise in pressure of 70 mm. of mercury. After 10 minutes 2 mgs. of infundibular extract given in the same way caused a rise of 30 mm. of mercury. One hour later, after 10 mgs. of infundibular extract had been given, a dose of 1 c. c. of 1 to 200,000 epinephrine caused a rise of 46 mm. of mercury whereas a 2 mg. dose of pituitary extract caused a rise in blood pressure of 40 mm. of mercury. In this experiment there was a decreased response to epinephrine and an increased response to infundibular extract. Furthermore, in some experiments the opposite result was noted, while in other experiments there was a marked decrease to infundibular extract and no change in the reaction to epinephrine. The change in reaction was apparently due to the state of anesthesia and to the amount of infundibular extract given to the animal. As the experiments with epinephrine did not justify using it as a standard, it was necessary to use infundibular extract itself. The infundibular extract for use as a standard was made from dried cattle material which had been collected at various times from local sources. It was obtained during the months of May, 1914, March, February, and July, 1915. Within a few minutes after killing the animals the pituitary bodies were removed and brought to the laboratory where the posterior lobe was carefully dissected free and minced and dried at a temperature of 60° C. for about 12 hours. The dried posterior lobe material was then finely pulverized in an agate mortar and used for making the standard solution, which was prepared in the following manner: One gram of the pulverized posterior lobe material was placed in a 300 c. c. Erlenmeyer flask, to which were added 95 c. c. of distilled water and 5 c. c. of 1 per cent acetic acid, and boiled for 10 minutes. After boiling it was filtered and made up to 100 c. c. One c. c. would be equivalent to 10 mg. of dried, or 50 mg. of fresh, material. The filtrate was clear and was acid to litmus. It was then put into 2 c. c. glass ampoules and sterilized at 100° C., on three successive days, for a daily period of 20 minutes.

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[1] By allowing rather long intervals between successive injections, greater accuracy can possibly be secured than when short intervals are allowed.

[2] The specimen of epinephrine chloride which was used in these experiments was made in the Hygienic Laboratory from commercial natural l epinephrine which by repurification yielded a fine crystalline substance whose physiological activity was several times that of the original material.

Isolated Uterus Method.—It has been shown that a simple and reliable method for comparing the activity of infundibular extracts is that on the isolated uterus of the virgin guinea pig. This method has been described in Hygienic Laboratory Bulletin No. 100, and was used in the present investigation in the following modified form. The animal was decapitated and the uterus quickly removed and placed in Locke's solution which was heated to about the body temperature. A 1 to 2 c. m. segment of one uterine horn was then removed from the vaginal end of the uterus. Silk threads were then tied into each end of the uterine segment, one of which was fastened to a stationary glass tube and the other to a straw writing lever, which was of such length that the tracing produced by it on a smoked drum was magnified about six times. Weights of from 1 to 4 grms. were added, to overcome the tonus into which the segment was thrown by the manipulation in arranging the segment for the experiment. The preparation was then submerged in a chamber of Locke's solution[1] which was maintained at a temperature of 39 degrees centigrade by an outer warming chamber, the temperature of which

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[1] Tyrode's solution has been successfully used by others.

was regulated by a bimetallic thermoregulator. The segment of uterus was always kept in 100 c. c. of the Locke's solution and supplied with oxygen at a uniform rate. After preparing the uterine segment in this way, spontaneous contractions will usually begin in about 15 to 30 minutes; occasionally they exceed these limits. The weights can then be readjusted, so as to secure a well-marked sweep of the lever. Instead of making the dilutions outside of the chamber containing the Locke's solution, as was recommended in Hygienic Laboratory Bulletin No. 100, the last dilution was made in the Locke's solution which contained the segment. This was done for the sake of simplicity, as it required less Locke's solution and less time to make the dilutions by the latter method. For example, if it were desired to ascertain the effect of a 1 to 10,000 solution of a certain preparation, 1 c. c. of a 1 to 100 solution of the preparation in Locke's was added to the chamber containing the segment, thereby making the desired solution. The chamber was emptied by means of a lower outlet tube and filled at the wide upper opening.

The standard previously recommended for the isolated uterus method was an aqueous solution of β-iminazolylethylamine hydrochloride in 1 to 20,000,000 concentration. In the present work, in addition to comparing the extracts with this standard, they were also compared with the standard solution of infundibular extract employed in the blood-pressure method, so that the same standard could be used for both methods.[1] A 1 to 1,000 stock solution of β-iminazolylethylamine hydrochloride (Hoffman-La Roche Co.) was made, using distilled water as the solvent, put into glass ampoules having a capacity of about 2 c. c., and sterilized at 100 degrees C. for 20-minute periods on three successive days.

YIELD OF POSTERIOR LOBE MATERIAL FROM VARIOUS MAMMALS.

The yield of fresh posterior lobe material from the various mammals may best be given in tabulated form. The weight of the anterior lobe will also be given, so as to show the relative yield of posterior lobe material from the pituitary body. The weighings were made as soon as possible after the material was collected and before drying had occurred. The large animals were killed, either by severing the large neck vessels or by having the brain crushed, and then quickly bled, while the small laboratory animals were etherized, or killed with illuminating gas, and then bled before the pituitary body was removed. In the small animals, it was impossible to separate the pars intermedia as a distinct entity. This was true to a certain degree in cattle. The colloid which was found in the space between the anterior and the posterior lobe was also carefully removed.

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[1] There was one exception to this rule. In series 7, only the "standard extract" was employed.

Table 1.—*Yield of anterior and posterior lobe material in cattle.*

Specimen No.	Weight of lobes in grms.		Ratio of weight of anterior to posterior.
	Anterior.	Posterior.	
1	3.215	0.507	6 to 1
2	2.441	.225	11 to 1
3	2.372	.332	7 to 1
4	1.681	.376	4 to 1

Table 2.—*Yield of anterior and posterior lobe material in sheep.*

Specimen No.	Weight of lobes in grms.		Ratio of weight of anterior to posterior.
	Anterior.	Posterior.	
1	0.323	0.023	14 to 1
2	.174	.013	13 to 1
3	.339	.019	18 to 1
4	.265	.028	9.5 to 1

Table 3.—*Yield of anterior and posterior lobe material in hogs.*

Specimen No.	Weight of lobes in grms.		Ratio of weight of anterior to posterior.
	Anterior.	Posterior.	
1	0.112	0.059	2 to 1
2	.140	.061	2.3 to 1
3	.140	.068	2 to 1
4	.150	.055	2.7 to 1

Table 4.—*Yield of anterior and posterior lobe material in cats.*

Specimen No.	Weight of lobes in grms.		Ratio of weight of anterior to posterior.
	Anterior.	Posterior.	
1	0.0157	0.009	1.7 to 1
2	.0130	.0085	1.5 to 1
3	.0165	.0065	2.5 to 1
4	.0148	.005	3 to 1
5	.0195	.009	2.1 to 1
6	.0148	.011	1.3 to 1
7	.020	.010	2 to 1

Table 5.—*Yield of anterior and posterior lobe material in rabbits.*

Specimen No.	Weight of lobes in grms.		Ratio of weight of anterior to posterior.
	Anterior.	Posterior.	
1	0.0255	0.0048	5.3 to 1
2	.0244	.009	2.7 to 1
3	.0195	.0045	4.3 to 1
4	.020	.005	4 to 1
5	.019	.0048	4 to 1

Table 6.—*Yield of anterior and posterior lobe material in dogs.*

Specimen No.	Weight of lobes in grms.		Ratio of weight of anterior to posterior.
	Anterior.	Posterior.	
1	0.015	0.008	2 to 1
2	.041	.019	2.2 to 1

Table 7.—*Yield of anterior and posterior lobe material in horses.*

Specimen No.	Weight of lobes in grms.		Ratio of weight of anterior to posterior.
	Anterior.	Posterior.	
1	1.505	0.385	4 to 1

MANNER OF MAKING EXPERIMENTAL INFUNDIBULAR EXTRACTS.

The experimental infundibular extracts used in this investigation were made from the fresh material in the following manner: After weighing the posterior lobe it was placed in an agate mortar and finely minced. It was then thoroughly triturated in the mortar and to the triturated material distilled water was added in amount equivalent to about 1 c. c. for every milligram of fresh material. This mixture was then transferred to an Erlenmeyer flask to which was added the rinsings from the mortar. For every 100 mg. of fresh material 5 c. c. of one-tenth per cent acetic acid were then added and the mixture boiled for 10 minutes. It was then filtered and, after filtration, distilled water was added so that each cubic centimeter represented the water soluble constituents from 1 mg. of fresh infundibular material.

EXAMINATION OF EXPERIMENTAL INFUNDIBULAR EXTRACTS MADE FROM FRESH MATERIAL.

After thus preparing the posterior lobes, they were examined physiologically by both the blood pressure and the isolated uterus methods, using as a standard for both methods the infundibular extract which was made in the laboratory from desiccated cattle material and which will be referred to as "standard extract." Besides the "standard extract," β-iminazolylethylamine hydrochloride was also used as a standard in the isolated uterus method in practically all cases. For the sake of brevity the latter will be referred to as β–I.

The experiments will be described in series as a rule. Each series required, for each method of assay, three or more animals; when two of the experiments gave similar results, only three experiments would be made in a given series.

Although the "standard extract" was made from desiccated material, the amounts mentioned in the following tables are in terms of fresh material, one part of the dried material being considered the equivalent of five parts of the fresh material, since it was found that in drying the posterior lobes in the manner described they would lose about 80 per cent in weight.

Series I.—In this series the infundibular extracts were made from the posterior lobe of the pituitary body of hogs, sheep, and cattle. They will be designated as extracts Hog I, Sheep I, Steer I, and Cow I. The examination of these extracts by the blood-pressure method gave the results recorded in the following table:

TABLE 8.

Infundibular extract examined.	Standard employed.	Amount of extract required to cause blood-pressure rise equivalent to "standard extract."
Hog I	2 mg. of "standard extract."	1.5 mg
Sheep I	do	2 mg.
Steer I	do	Do.
Cow I	do	Do.

The blood-pressure rise resulting from the intravenous administration to dogs of 2 mg. of "standard extract" depended largely upon the degree of anesthesia and varied from 15 to 55 mm. of mercury. As a check upon these doses double the amount was given.

In the next table is tabulated the relative activity of these extracts upon the isolated uterus of the virgin guinea pig. They were compared both with β I in 1 to 20,000,000 dilution and with the "standard extract"; the latter, in 1 to 500,000 dilution, having an effect equivalent to β–I in 1 to 20,000,000 dilution.

TABLE 9.

Infundibular extract examined.	Standards employed.		Concentration of extract required to cause a uterine response equivalent to that caused by standards (*a*), (*b*).
Hog I	(*a*) β–I in 1 to 20,000,000 dilution.	(*b*) "Standard extract" in 1 to 500,000 dilution.	1 to 2,000,000.
Sheep I	do	do	1 to 1,000,000.
Steer I	do	do	Do.
Cow I	do	do	Do.

Series 2.—The infundibular extracts used in this series were made from pituitary bodies obtained from young cattle and hogs. They will be designated as extracts Hog 2, Steer 2, and Steer 3. Examined by the blood pressure method, their activity is shown in Table 10.

TABLE 10.

Infundibular extract examined.	Standards employed.	Amount of extract required to cause blood pressure rise equivalent to standard extract.
Hog 2	2 mg. of "standard extract."	1 mg.
Steer 2	do	1.4 mg.
Steer 3	do	Do.

The relative activity of this series upon the isolated uterus of the virgin guinea pig is shown in Table 11.

TABLE 11.

Infundibular extract examined.	Standards employed.		Concentration of extract required to cause a uterine response equivalent to that caused by standards (*a*), (*b*).
Hog 2	(*a*) β-I in 1 to 20,000,000 dilution.	(*b*) "Standard extract" in 1 to 500,000 dilution.	1 to 1,000,000.
Steer 2	do	do	1 to 500,000.
Steer 3	do	do	Do.

Series 3.—This series comprised infundibular extracts made from cattle and sheep. They will be named extracts Steer 4 and Sheep 2. By the blood-pressure method they were found to possess the following relative activity:

TABLE 12.

Infundibular extract examined.	Standards employed.	Amount of extract required to cause blood pressure rise equivalent to standard extract.
Steer 4	2 mg. "standard extract".	2 mg.
Sheep 2	do	Do.

Their activity on the isolated uterus of the virgin guinea pig is shown in Table 13.

TABLE 13.

Infundibular extract examined.	Standards employed.		Concentration of extract required to cause a uterine response equivalent to that caused by standards (*a*), (*b*).
Steer 4	(*a*) β I in 1 to 20,000,000 dilution.	(*b*) "Standard extract" in 1 to 500,000 dilution.	1 to 500,000.
Sheep 2	do	do	1 to 1,000,000.

Series 4.—An infundibular extract, made from the pituitary body obtained from an old horse, and one made from the pituitary body obtained from a steer, were examined in this series. The extracts are

designated horse extract and steer 5. Their comparative activity, by the blood-pressure method, is shown by table 14.

TABLE 14.

Infundibular extract examined.	Standard employed.	Amount of extract required to cause blood pressure rise equivalent to "standard extract."
Horse	2 mg. "standard extract"	1.3 mg.
Steer 5	do	Do.

The following table shows their activity on the isolated uterus of the virgin guinea pig:

TABLE 15.

Infundibular extract examined.	Standards employed.		Concentration of extract required to cause a uterine response equivalent to that caused by standards (*a*), (*b*).
Horse	(*a*) β-I in 1 to 20,000,000 dilution.	(*b*) "Standard extract" in 1 to 500,000 dilution.	1 to 1,000,000.
Steer 5	do	do	Do.

Series 5.—In this series infundibular extracts made from the pituitary body of a cat and of a dog were examined. They will be designated Cat 1 and Dog 1. Their activity, by the blood-pressure method, is shown in the following table:

TABLE 16.

Infundibular extract examined.	Standard employed.	Amount of extract required to cause blood pressure rise equivalent to "standard extract."
Cat 1	2 mg. "standard extract"	0.5 mg.
Dog 1	do	Do.

Their activity, by the isolated-uterus method, is shown in table 17.

TABLE 17.

Infundibular extract examined.	Standards employed.		Concentration of extract required to cause a uterine response equivalent to that caused by standards (*a*), (*b*).
Cat 1	(*a*) β-I in 1 to 20,000,000 dilution.	(*b*) "Standard extract" in 1 to 500,000 dilution.	1 to 2,000,000.
Dog 1	do	do	Do.

Series 6.—Infundibular extracts from rabbits and cats comprise this series. The posterior lobes from two full-grown female rabbits and from two full-grown male cats were used in making the extracts.

They will be designated Rabbit 1 and Cat 2. These extracts were examined by the blood-pressure method, using not only the dog as the test animal, but the cat and the rabbit as well. With the exception of one rabbit, the comparative results in all of the test animals used in the blood-pressure method were the same. In this rabbit the results differed in degree. Two mg. of "standard extract" were equivalent to 0.5 mg. of cat extract; whereas both these extracts in the above doses were only slightly more active than 0.5 mg. of rabbit extract. This would suggest that certain rabbits are more sensitive to extracts made from the pituitary bodies obtained from rabbits than to extracts derived from other animals. The results of the remaining experiments are given in table 18.

TABLE 18.

Infundibular extract examined.	Standard employed.	Amount of extract required to cause blood-pressure rise equivalent to "standard extract."
Rabbit 1	2 mg. "standard extract".	2 mg.
Cat 2	do	0.5 mg.

In the following table is tabulated their relative activity on the isolated uterus of the virgin guinea pig:

TABLE 19.

Infundibular extract examined.	Standards employed.		Concentration of extract required to cause a uterine response equivalent to that caused by standards (*a*), (*b*).
Rabbit 1	(*a*) β-I in 1 to 20,000,000 dilution.	(*b*) "Standard extract" in 1 to 500,000 dilution.	1 to 1,000,000.
Cat 2	do	do	1 to 2,000,000.

Series 7.—The pituitary bodies obtained from three male rabbits, three male cats, and two dogs were used in making the infundibular extracts examined in this series. The extracts will be designated Rabbit 2, Cat 3, and Dog 2. For the examination of the activity of these extracts on the blood pressure, the dog, the rabbit, and the cat were used as test animals. The result, by the blood-pressure method, is shown in the following table:

TABLE 20.

Infundibular extract examined.	Standard employed.	Amount of extract required to cause blood-pressure rise equivalent to "standard extract."
Rabbit 2	2 mg. "standard extract".	1 mg.
Cat 3	do	0.5 mg.
Dog 2	do	Do.

The next table will show their activity on the isolated uterus of the virgin guinea pig.

TABLE 21.

Infundibular extract examined.	Standard employed.	Concentration of extract required to cause a uterine response equivalent to that caused by the standard.
Rabbit 2	"Standard extract" in 1 to 500,000 dilution.	1 to 500,000.
Cat 3	do	1 to 2,000,000.
Dog 2	do	Do.

Summary of results obtained in series 1 to 7.—From the results obtained in series 1 to 7 it may be concluded that of the extracts examined the infundibular extracts made from pituitary bodies of cats and dogs possess the highest degree of activity, the hog extracts being somewhat less active than those made from cats or dogs; whereas sheep extract, rabbit extract, horse extract, and cattle extract are about equally active. Individual variation occurs, and is especially shown in the various cattle extracts examined, their degree of variation being in certain instances 100 per cent.

EFFECT OF CERTAIN PROCEDURES ON ACTIVITY OF INFUNDIBULAR EXTRACTS.

Effect of addition of acetic acid.—Several experiments were carried out to determine whether infundibular extracts made with acetic acid varied in activity from those made without acetic acid, and it was found that there was no difference. The addition of acetic acid to the water containing the infundibular material is necessary, however, for the proper filtration of the soluble constituents.

Effect of drying fresh infundibular material.—Drying the fresh infundibular material at from 50 to 60° C. for from 12 to 15 hours resulted in no loss of activity. Higher temperatures than these were not tried.

Extraction of active substances from infundibular material, by maceration in water acidulated with acetic acid.—The active substances in fresh infundibular material are extracted partially by maceration for 12 to 15 hours in water acidulated with acetic acid; whereas they are extracted more completely by boiling for a period of 10 minutes.

COMMERCIAL INFUNDIBULAR EXTRACTS EXAMINED.

Seven samples of commercial infundibular extracts, the product of five American manufacturing pharmacists, were examined by the blood pressure and by the isolated uterus methods. They will be referred to as samples 1 to 7, inclusive. All of them were bought on the open market, from local sources, by the laboratory pharmacist. Samples 1 to 4, inclusive, were bought in May, 1915; samples 5 to 7, inclusive, were purchased in December, 1915. The examination of the commercial infundibular extracts was made at the following times: Samples 1 and 2 in October, 1915; samples 3 and 4 in November, 1915; samples 5, 6, and 7 in January, 1916. Sample 2 was again examined in January, 1916. They were dispensed in 1 cc. glass ampoules. With the exception of samples 3 and 6, which were sterile solutions only, all were stated to contain, as a preservative, chlorbutanol in quantities of 5 mg. per cubic centimeter. In the following table the trade name, manufacturer's name, and serial number of these extracts will be given:

TABLE 22.

Sample No.	Trade name.	Manufacturer.	Serial No.
1	Pituitary extract	Eli Lilly & Co.	24973 514522
2	Pituitrin	Parke, Davis & Co.	1900871
3	Pituitary extract	H. K. Mulford Co.	23742 231299A
4	.....do	The Norwich Pharmacal Co.	27149 32915
5	.....do	.....do	31549
6	Pituitary liquid	Armour & Co.	500028
7	Pituitary extract	Eli Lilly & Co.	165930 542184

No statement was made, either on the labels or in the advertising literature of samples 1, 3, and 4, as to when the preparations should not be used. The labels of the remaining samples, however, contained statements which cautioned against the use of the preparation after a certain date, the limit at which full activity was guaranteed for any one not extending beyond December, 1916.

Activity of these commercial infundibular extracts.—In Table 23 their relative activity on the blood pressure is shown.

TABLE 23.

Sample No.	Standard employed.	Amount of extract required to cause blood pressure rise equivalent to Standard extract.	Relative activity.
1	2 mg. of "Standard extract"	8 cc. of 1 to 25 dilution	1
2	.....do	2 cc. of 1 to 50 dilution	8
3	.....do	.....do	8
4	.....do	6 cc. of 1 to 50 dilution	3
5	.....do	2 cc. of 1 to 10 dilution	5
6	.....do	2 cc. of 1 to 50 dilution	8
7	.....do	.....do	8

Their activity, as determined by the isolated uterus method, is shown in the following table:

TABLE 24.

Sample No.	Standard employed.		Concentration of extract required to cause a uterine response equivalent to that caused by standards (a), (b).	Relative activity.
1	(a) β I in 1 to 20,000,000 dilution.	(b) "Standard extract" in 1 to 500,000 dilution.	1 to 2,000	1
2	do	do	1 to 20,000	10
3	do	do	1 to 20,000	10
4	do	do	1 to 5,000	2.5
5	do	do	1 to 4,000	2
6	do	do	1 to 20,000	10
7	do	do	1 to 20,000	10

From the above tables it is seen that, by both of the above methods, samples 2, 3, 6, and 7 are equally active physiologically, and are many times more active than the remaining samples. As has been previously stated, samples 5 and 7 were bought later than samples 1 and 4. Relatively little difference in activity was found in samples 4 and 5, which are the product of the same manufacturer, whereas sample 7 is many times more active than sample 1, both of which were made by the same manufacturer. It is probable that the initial strength of samples 4 and 5 was relatively low, while sample 1 may have undergone deterioration. A second examination of sample 2 was made at the conclusion of these experiments, to ascertain whether any deterioration had occurred in the standards. It was found that the relative activity of the standards and of sample 2 was exactly the same as was found at the first examination. It was, therefore, considered that there was no alteration in the activity of the standards during the course of this investigation.

Effect of commercial infundibular extracts on isolated intestine of rabbit.—In the standardization of sample 7, it was discovered that while 2 c. c. of a 1 to 50 dilution would cause the same rise in the blood pressure as 2 mg. of the standard extract, double the amounts would not yield the same relative results; that is, 4 mg. of the standard extract was more active than 4 c. c. of a 1 to 50 dilution of sample 7. It appeared, therefore, that the substance which is normally present in infundibular extracts and which depresses the circulation was not perceptible in small doses, while in large doses it produced significant effects. That this phenomenon was not due to the preservative chlorbutanol, is attested to by the fact that it was the only one of the five preparations containing this preservative which behaved in this manner. Inasmuch as Shamoff (1916) and Hoskins (1916) have recently shown that certain infundibular extracts contain a substance which induces depression of intestinal motility while others induce pure stimulation, it was thought that sample 7 might possibly contain a substance which had a depressant effect on the isolated intestine. This question was accordingly investigated, using the isolated intestine of the rabbit as the test organ. The rabbits which were used for this purpose were anesthetized with urethane (1.5 to 2 grms. per kilo by the stomach), after which a 2 to 3 cm. segment of intestine was removed from the lower part of the ileum, immersed in Locke's solution at 38 degrees Centigrade, and arranged in the usual way for recording its movements. It was found that a 1 to 200 dilution of sample 7 produced a marked increase in the height of the intestinal contractions, and a decrease in the extent of relaxation. This was followed in 2 to 3 minutes by irregularities, some slowing, but no relaxation below normal, the individual contractions being almost the height of the normal contractions. If the dilution of sample 7 were increased to 1 in 40; the intestinal movements were at once inhibited and relaxation, to a degree slightly below normal, occurred. Inasmuch as sample 7 contained chlorbutanol, a similar amount of chlorbutanol itself was tried and marked inhibition, together with relaxation below normal, occurred—results which were very similar to those obtained when using a 1 to 40 dilution of sample 7.

These experiments suggested that the preservative was the cause of the inhibition and the relaxation which were obtained when using a 1 to 40 dilution of sample 7. The following infundibular extracts, which were free from preservative, were then tried—namely, "standard extract" in 1 to 200 dilution; infundibular extract which was made from fresh cattle material (Armour & Co.),[1] using 1 to 200 dilution; and infundibular extract which was made from cattle material that had been desiccated in vacuo at 35 to 40° C. (Armour & Co.), using 1 to 200 and 1 to 40 dilutions. Without exception, all of these extracts which contained no preservative produced an increase in the extent of the contraction and a decrease in the degree of the relaxation. Occasionally, when using infundibular extract made from fresh material, a slight lessening in the extent of contraction occurred immediately after changing to the infundibular extract. This effect was not constant and, when present, lasted for only about 10 to 30 seconds. At no time was relaxation below normal noted when preparations free from preservatives were used. Although these experiments do not answer the question for which they were intended, they do show that the preservative, chlorbutanol, is a disturbing factor in the examination of infundibular extracts by an intestinal method, especially when concentrated solutions are used. In this connection it should be stated that the name "Pituitrin" has sometimes inappropriately been given by certain writers to infundibular extracts other than this particular commercial preparation. Since "Pituitrin" contains chlorbutanol as a preservative, it is not strictly comparable to infundibular extract. No doubt some of the conflicting statements in regard to the pharmacological action of infundibular extracts can be explained in this way.

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[1] These infundibular extracts were kindly furnished me by Mr. Frederic Fenger of Armour & Co.

DISCUSSION OF FOREGOING RESULTS.

The difference in the ratio between the relative activity of the various experimental infundibular extracts, by the isolated uterus and by the blood pressure methods, emphasizes the fact that both methods should be employed to determine the physiological activity of preparations of this type, as it has been particularly shown in series 3 that by one method certain preparations may be equal in activity and by the other method one was double the activity of the other. If the blood pressure method is also to be employed, a very important question to be considered in that connection is the standard to be adopted. If the response to epinephrine were similar at all times to that caused by infundibular extracts, it would be a more satisfactory standard than a preparation of infundibular extract; since the circulatory effect of epinephrine is quite similar to that caused by infundibular extract and, in addition, the epinephrine response is a very fleeting one. On the other hand, if a preparation of infundibular extract were to be used, it should be employed in relatively small doses only, so that the animal will not quickly become saturated with the extract, thus avoiding the production of the marked depressor effect on the circulation which practically always occurs after several large doses are given. It would seem that a working standard, which would be applicable to both methods, could be had in desiccated cattle infundibular material, since it is considered to be a comparatively stable substance. The employment of standards of unequal activity by the various supply houses could easily be eliminated by having a central laboratory distribute the desiccated cattle infundibular material for use as a standard. If the standardization of infundibular extracts is to be limited to a determination of but one of its physiological effects, we believe that the effect on the uterus should be taken as an index of its activity, on account of the fact that these extracts are mainly used as oxytoxics, and that the histidine derivative β-iminazolylethylamine hydrochloride should be used as the standard.

From a practical standpoint the selection of cattle infundibular material as the source of infundibular extracts has been well made. However, if horse and hog infundibular material is more readily secured in some localities than is cattle material, these sources should be taken advantage of in such instances for commercial reasons.

As is seen from the tables above, infundibular extracts made from the pituitary bodies of cats, dogs, or hogs are more active than similar extracts made from the pituitary bodies of cattle, sheep, horses, and rabbits. This would suggest that the character of the food consumed by animals influences the activity of the infundibular lobe of the pituitary body, and that extracts made from infundibular material obtained from carnivorous and omnivorous animals are more active than those from herbivorous animals.

The observations of Herring (1914), in which he found that extracts made from the pars nervosa of cattle were from two to five times as active on the uterus as extracts from the pars intermedia, and that the pars intermedia had no specific effect on the blood pressure, whereas the pars nervosa was mainly responsible for the pressor effect, should be considered in discussing the probable causes of species variation. The individual variation which was found in the same species, and which is particularly well illustrated by the variation in the cattle extracts examined, may be due, in a measure, to a possibly incomplete separation of the pars intermedia from the pars nervosa.

SUMMARY.

The determination of the activity of infundibular extracts made from the pituitary body obtained from various species of mammals—namely, cattle, sheep, horses, hogs, rabbits, cats, and dogs—was made by both the blood pressure and the isolated uterus methods, and it was found that the infundibular extracts made from cats or dogs were more active than similar extracts made from the other species of mammals. An individual variation was shown in extracts made from the same species of mammals. Commercial infundibular extracts examined in the same way showed a variation of 800 per cent by the blood pressure method and of 1,000 per cent by the isolated uterus method. A commercial preparation of infundibular extract which contained chlorbutanol as a preservative was shown to depress the motility of the isolated intestine of the rabbit when used in concentrated solution.

CONCLUSIONS.

1. The activity of infundibular extracts on the isolated uterus of the virgin guinea pig should not be taken as an index of their activity on the blood pressure.

2. Infundibular extracts made from the pituitary bodies obtained from cattle, horses, hogs, cats, dogs, and rabbits differ quantitatively in their activity.

3. The infundibular lobe of the pituitary body of cats, dogs, and hogs contains a greater amount of active materials than from cattle, sheep, horses, or rabbits.

4. Commercial infundibular extracts vary widely in their physiological activity.

5. The motor depression of the isolated intestine which is caused by certain commercial infundibular extracts is due in certain instances to the depressing effect of the preservative chlorbutanol.

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PHARMACOLOGICAL STUDIES WITH COCAINE AND NOVOCAINE. A COMPARATIVE INVESTIGATION OF THESE SUBSTANCES IN INTACT ANIMALS AND ON ISOLATED ORGANS.[1]

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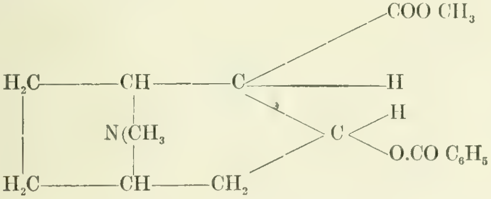
INTRODUCTION.

Soon after the discovery of cocaine and the announcement that it possessed anesthetic properties, intensive studies were made to determine the relation between its chemical constitution and the production of anesthesia. It was early recognized that cocaine was a dangerous therapeutic agent, and in the investigations that were conducted with the view of ascertaining the relation between its chemical nature and its anesthetic qualities, other compounds were discovered which produced anesthesia and were less toxic, but in many cases were too irritant or possessed some other undesirable properties which prohibited their use as local anesthetic agents. Two distinct conceptions were brought out by the early investigators with cocaine to explain the anesthesia which it and its derivatives produce. Cocaine may be resolved into the base ecgonine, methyl alcohol, and benzoic acid. It is represented by the following formula (Meyer and Gottlieb, 1914):



Cocaine (Benzoyl-methyl-ecgonine).

Stockman, 1886, investigated benzoyl-ecgonine physiologically, and found that it did not paralyze nerve endings in a manner similar to cocaine. He expressed the opinion that the relationship between the action of cocaine and benzoyl-ecgonine was altered by the substitution of the methyl-group.

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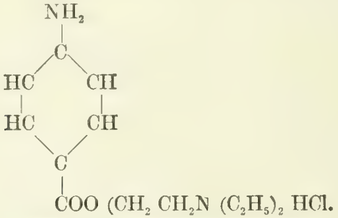
Filehne, 1887, found that methyl-ecgonine was incapable of producing anæsthesia when applied locally, and considered that the benzoyl radical was the essential factor in imparting such anesthetic properties to cocaine.

Poulsson, 1890, regarded esterification with certain aliphatic radicals, such as a methyl, ethyl, or propyl radical, as the most important procedure in producing compounds of the local anesthetic type.

Since the investigations of Stockman, 1886, Filehne, 1887, and Poulsson, 1890, many derivatives of benzoic acid have been discovered which have the properties of a local anesthetic, among them being a compound, the hydrochloride of which has been given the name of novocaine. According to Meyer and Gottlieb, 1914, is has the following structural formula:



Novocaine (ρ-amido-benzoyl-diethyl-amidoethanol hydrochloride).

Novocaine hydrochloride occurs as a white substance which is made up of small crystals soluble in one part of water and in 30 parts of alcohol. It melts at 156° C. Its aqueous solution is neutral, from which it can be precipitated by all the common alkaloidal reagents.

At the present time novocaine is perhaps one of the most widely used agents for producing local anesthesia. Its very general use is dependent largely upon the fact that it is usually considered to be a comparatively safe and efficient local anesthetic; also because it has a wider range of application since, besides being readily soluble in water, it is said to withstand heating to comparatively high temperatures.

An analysis of the statements in the textbooks on pharmacology and of the original literature has shown that there is no general agreement among the various authors or the various investigators as to the exact toxicity of novocaine. This toxicity is usually expressed in terms of cocaine toxicity. Halsey, 1914, states that novocaine is one-half as toxic as cocaine. According to Cushny, 1915, novocaine is one-third as toxic as cocaine, while Meyer and Gottlieb, 1914, regard the ratio of toxicity between cocaine and novocaine to be as 1 is to 0.3. Biberfeld, 1905, concluded that novocaine was one-fifth to one-sixth as toxic as cocaine. Le Brocq, 1909, found cocaine to be about twice as toxic as novocaine. Piquand and Dreyfus, 1910, concluded that novocaine was one-fourth to one-sixth as toxic as cocaine. Marshall, 1911, found that the relative toxicity of cocaine and novocaine was approximately as 1 is to 0.3.

It is evident from the above statements that the question of the relative toxicity of novocaine and cocaine is unsettled. The analysis of the literature further discloses the fact that although the toxicity of novocaine is relatively low, like cocaine, it is sometimes productive of untoward results, death being attributed to it in certain instances.

In consideration of the lack of uniformity in the statements regarding the toxicity of novocaine, the fact that in some cases it has produced untoward results, and for the reason that the pharmacological studies have been limited mainly to a determination of the toxicity of novocaine in intact animals, it was thought desirable to investigate the action of novocaine in order to determine not only its toxicity in intact animals, but to ascertain its effect upon the blood pressure and respiration as well as upon the heart and certain other body tissues. Such knowledge might possibly offer some explanation for the untoward results which have been reported from the use of novocaine in man. As in previous investigations, for the purpose of comparison with a well-known substance which is similarly used, comparison was made with cocaine.

The report of the studies made of these preparations is dealt with in five parts, namely: (1) Toxicity in intact animals; (2) action on heart muscle; (3) action on smooth muscle; (4) effect on the blood pressure and respiration; (5) general considerations.

PART I.

TOXICITY IN INTACT ANIMALS.

In the following experiments the toxicity of cocaine and novocaine was determined by ascertaining the minimal dose required to produce death within 24 hours when given subcutaneously or intravenously. The minimal dose which was fatal was determined in frogs, mice, rats, guinea pigs, and rabbits.

The cocaine hydrochloride which was used for most of the toxicity experiments was made by C. A. F. Kahlbaum. It was obtained in its original package and was unopened when received. When heated to 189.1° C. it liquefied as a dark-brown substance. A small amount of cocaine hydrochloride, which was obtained from the Hoffmann-

La Roche Chemical Works, was also used. This preparation liquefied at 189° C. The third sample of cocaine hydrochloride used was a Merck & Co. preparation. It liquefied at 190.2° C.

A number of samples of novocaine were employed for determining the minimal lethal doses. They were obtained upon the open market, and were in containers labeled with the manufacturer's name. Some of the bottles had been opened, part of the contents of the bottles having been previously removed.

The melting point of each sample was taken and it was found that the various samples agreed very closely as regards the melting point. The various results are recorded in the following table.

TABLE 1.—*Melting point of samples of cocaine and novocaine.*

Serial No. of sample.	Melting point.	How received.
800	155-156.5° C.	Unopened.
671	155-155.2° C.	Do.
644	155-156.5° C.	Opened.
513	154-156.5° C.	Do.
25066[1]	153-156° C.	Not in original bottle.
614	155-156.5° C.	Unopened.
16	153-157° C.	Opened.
772	153-156.5° C.	Do.
707	153-156.2° C.	Do.
219	153-156° C.	Unopened.

[1] Druggist's number.

Most of the experiments with novocaine were done with sample No. 800.

TABLE 2.—*Toxicity of cocaine hydrochloride in frogs* (*Rana pipiens*) *when injected into ventral lymph sac.*

Frog No.	Sex.[1]	Weight in gms.	Dose in mgs. per gm. body weight.	Result.[2]	Remarks.
25	M	25	0.05	—	
20	M	25	0.1	—	
26	M	25	0.1	—	
4	M	31	0.2	—	
19	M	25	0.3	—	
1	M	23	0.5	—	
27	M	27	0.5	—	
18	M	23	0.7	+	Died after 3 days.
2	M	32	1.0	—	Do.
13	M	30	1.0	—	
28	F	28	1.0	+	Died within 12 hours.
14	M	27	1.5	?	
3	M	32	1.5	+	Died within 7 hours.
17	M	22	1.5	+	Died within 24 hours.
15	M	27	2.0	+	Do.
16	M	27	3.0	+	Do.

[1] Male=M; Female=F.

[2] Survived="—"; died="+".

M. L. D.=1 mg. per gm. of body weight or 1 gm. per kilo of body weight. However, 0.7 mg. per gm. o body weight killed in 3 days.

Table 3.—*Toxicity of novocaine hydrochloride in frogs (Rana pipiens) when injected into ventral lymph sac.*

Frog No.	Sex.[1]	Weight in gms.	Dose in mgs. per gm. body weight.	Result.[2]	Remarks.
24	F	35	0.1	–	
23	F	31	0.2	–	
8	M	25	0.4	–	Died after 7 days.
29	F	32	0.4	–	
22	M	30	0.5	–	
30	M	32	0.7	+	Died within 12 hours.
31	F	33	1.0	+	Do.
5	M	31	1.0	+	Died in about 7 hours.
9	M	33	1.0	–	
32	M	35	1.2	+	Died within 24 hours.
10	M	33	1.5	+	Do.
21	M	28	1.5	+	Do.
6	F	29	2.0	+	Do.
11	M	32	2.0	+	Do.
7	M	26	3.0	+	Died after 7 hours.
12	F	30	3.0	+	Died within 24 hours.

[1] Male="M."; female="F."
[2] Survived="–"; died="+."

Toxicity· M. L. D.=0.7 mg. per gm. body weight or 0.700 gm. per kilo body weight.

In frogs, if the dose of cocaine were sufficiently small, a period of excitement followed the depression. With larger doses of cocaine the excitement stage was absent. Depression usually occurred, regardless of the dosage, when novocaine was given.

Table 4.—*Toxicity of cocaine hydrochloride in white mice administered subcutaneously.*

Mouse No.	Sex.[1]	Weight in gms.	Dose in gms. per gm. body weight.	Dose in gms. per 20 gms. body weight.	Result.[2]	Remarks.
84	M	22.16	0.00005	0.001	–	No effect.
85	F	22.30	0.00007	0.0014	–	
86	M	20.67	0.00009	0.0018	–	Died after 5 days.
87	M	27.74	0.0001	0.002	–	
88	M	20.75	0.0001	0.002	+	Died after 12 hours.
65	M	22.06	0.0001	0.002	–	No effect.
66	M	21.4	0.00015	0.003	+	Died within 12 hours.
89	M	23.35	0.00015	0.003	+	Died within 6 hours.
90	M	29.35	0.0002	0.004	+	Do.
67	M	19.76	0.0002	0.004	+	Died within 3 hours.
68	F	21.55	0.00025	0.005	+	Do.
91	F	26.85	0.00025	0.005	+	Do.
69	M	24.14	0.0003	0.006	+	Do.
70	M	20.25	0.00035	0.007	+	Do.
71	M	23.15	0.0004	0.008	+	Do.
64	F	19.25	0.0005	0.010	+	Do.

[1] Male="M"; female="F". [2] Survived="–"; died="+".

Toxicity: M. L. D.=0.0001; S. L. D.=0.00015 (surely lethal dose).

TABLE 5.—*Toxicity of novocaine hydrochloride in white mice administered subcutaneously.*

Mouse No.	Sex.[1]	Weight in gms.	Dose in gms. per gm. body weight.	Dose in gms. per 20 gms. body weight.	Result.[2]	Remarks.
56	M	23.5	0.0002	0.004	–	
57	M	21.82	0.0003	0.006	–	
92	M	18.33	0.0003	0.006	–	
58	F	23.26	0.0004	0.008	–	
93	F	20.83	0.0004	0.008	–	
59	M	22.25	0.0005	0.010	–	
94	M	20.6	0.0005	0.010	–	
95	F	19.38	0.00055	0.011	+	Died within 1 hour.
96	M	19	0.0006	0.012	+	Do.
97	F	24.32	0.0006	0.012	+	Do.
60	M	22.34	0.0006	0.012	+	Died after 1½ hours.
98	F	25.95	0.00065	0.013	–	Pregnant animal.
61	M	20.12	0.0007	0.014	+	Died after 1½ hours.
99	M	22.25	0.0007	0.014	+	Died within 1 hour.
62	M	21.17	0.0008	0.016	+	Died after ¾ hour.
63	M	21.6	0.001	0.020	+	Do.

[1] Male="M"; female="F".

[2] Survived="–"; died="+".

Toxicity: M. L. D.=0.550 gm. per kilo. of body weight. It is of interest to note that the animal which survived a lethal dose was pregnant.

The symptoms produced in mice, from the subcutaneous administration of cocaine, were unlike those produced by novocaine. Stimulation was invariably produced by cocaine; animals which received twice the minimal lethal dose, would become very much excited in about 10 or 15 minutes, and would run about wildly. The respiration and reflex irritability were increased in about one-half hour after the injection, alternate periods of clonic convulsions and quiet would intervene. This condition would last for several hours, when death would occur, apparently from exhaustion.

The symptoms caused in white mice by the subcutaneous administration of a M. L. D. of novocaine hydrochloride were drowsiness, decrease in respiration after a short period of increased respiration, muscular weakness, difficulty in walking, and finally, inability to walk. At no time were convulsions present.

TABLE 6.—*Toxicity of cocaine in white rats when given subcutaneously.*

Rat No.	Sex.[1]	Weight in gms.	Dose in mgs. per kilo body weight.	Result.[2]	Remarks.
10	M	265	25	−	
9	M	243	50	−	
8	F	193	75	−	
7	F	161	100	−	
17	M	285	100	−	
16	M	180	150	−	Died after 36 days. Lung disease.
6	M	176	200	+	Died after 15 hours.
20	F	215	200	−	
18	M	213	250	+	Died after 20 hours.
38	M	235	275	−	
19	F	200	300	−	Died after 25 days. Lung disease.
36	M	202	300	+	Died within 24 hours.
35	M	175	350	−	
34	M	150	375	+	Do.
41	F	195	375	+	Died within 22 hours.
43	M	247	375	+	Do.
25	F	150	400	+	Died within 5½ hours.
37	M	145	400	+	Died within 24 hours.
33	M	180	400	+	Do.
26	F	157	500	+	Died within 14 hours.
27	F	187	600	+	Died within 18½ hours.
28	F	185	700	+	Died within 17½ hours.

[1] Male="M"; female="F."

[2] Survived="−"; died="+."

Toxicity: M. L. D.=200 mg.; S. L. D. (surely lethal dose) =375 mg.

TABLE 7.—*Toxicity of novocaine in white rats when given subcutaneously.*

Rat No.	Sex.[1]	Weight in gms.	Dose in mgs. per kilo body weight.	Result.[2]	Remarks.
5	M	276	50	−	
4	M	186	75	−	Died after 26 days. Lung disease.
3	F	167	100	−	
2	M	195	200	−	
13	M	243	300	−	Died after 31 days. Lung disease.
14	F	220	400	−	Died after 18 days.
1	M	195	400	−	
12	M	205	600	−	Died after 28 days. Lung disease.
15	F	190	800	−	Died after 2½ months. Lung disease.
11	M	180	1,000	−	Died after 33 days. Lung disease.
31	M	180	1,000	−	
28	M	162	1,250	−	
30	F	157	1,500	+	Died within 12 minutes. Dose given intraperitoneally by mistake.
32	M	137	1,750	−	
40	M	198	1,750	−	
44	M	157	2,000	−	Died after 43 days.
21	F	165	2,000	+	Died after 2½ days.
42	M	150	2,250	−	Died after 6 days.
39	F	137	2,500	+	Died after 5 hours.
22	F	172	3,000	+	Died after 2¼ hours.
23	F	170	4,000	+	Died after 5½ hours.
24	M	163	5,000	+	Do.

[1] Male="M"; female="F."

[2] Survived="−"; died="+."

Toxicity: M. L. D.=2,000 mg. per kilo of body weight.

The symptoms produced by cocaine in white rats were not especially pronounced. There was a period of mild excitement preceding the final depression. Novocaine produced depression only.

TABLE 8.—*Toxicity of cocaine hydrochloride in guinea pigs when administered subcutaneously.*

Guinea pig No.	Sex.[1]	Weight in gms.	Dose in mgs. per kilo body weight.	Result.[2]	Remarks.
13	F	220	20	–	No symptoms.
5	M	243	30	–	Few symptoms; gritting teeth for about 1 hour.
14	F	220	40	–	Clonic convulsions, walking about after 40 minutes.
15	M	225	40	–	
6	M	245	50	–	
16	F	225	55	–	
12	F	445	60	+	Dead after 20 minutes.
7	M	250	60	+	Dead after 12 minutes.
8	M	240	70	+	Dead after 30 minutes.
9	F	375	80	+	
10	F	380	90	+	
11	F	395	100	+	Dead after 18 minutes.

[1] Male="M"; female="F."

[2] Survived="–"; died="+."

Toxicity: M. L. D.=60 mg. per kilo of body weight.

TABLE 9.—*Toxicity of novocaine hydrochloride in guinea pigs when administered subcutaneously.*

Guinea pig No.	Sex.[1]	Weight in gms.	Dose in mgs. per kilo body weight.	Result.[2]	Remarks.
23	M	290	200	–	Depression. Recovery in 3 hours.
24	M	285	300	–	
26	M	285	400	–	
25	M	280	500	–	Died after 3 days. Cause.
1	M	285	500	–	
27	M	310	600	+	Died after 17 minutes.
2	M	240	600	+	Died after 23 minutes.
28	M	325	700	+	Died after 32 minutes.
3	M	260	800	–	Died on 4th day from infection.
29	M	325	800	+	Died after 20 minutes.
22	M	240	900	+	Died after 3 hours.
20	M	225	1,000	+	Died after 25 minutes.
4	M	260	1,000	–	Died on the 4th day from infection.
21	M	250	1,000	+	Died after 20 minutes.
17	F	225	1,200	+	Died after 30 minutes.
18	F	220	1,500	+	Died after 20 minutes.
19	M	220	1,700	+	Died after 7 minutes.

[1] Male="M"; female="F."

[2] Survived="–"; died="+."

Toxicity: M. L. D.=0.600 gm. per kilo of body weight.

In guinea pigs a lethal dose of novocaine would usually cause muscular weakness, together with considerable decrease in the rate and depth of respiration, within 5 to 10 minutes after its subcutaneous injection. About 20 minutes after the injection death would occur, which was often preceded by a series of abortive clonic type convulsions. After a lethal dose of cocaine guinea pigs would become very much excited and would run about wildly. The respiration and the reflexes were increased. Tonic and clonic convulsions then followed every few seconds until death supervened.

TABLE 10.—*Toxicity of cocaine hydrochloride in rabbits when administered subcutaneously.*

Rabbit No.	Sex.[1]	Weight in kilos.	Dose in mgs. per kilo body weight.	Result.[2]	Remarks.
34	M	2.6	20	–	
35	M	2.3	30	–	
36	M	2.35	40	–	
12	M	2.3	50	–	
37	M	2.3	60	–	
5	F	1.85	75	+	Died after 45 minutes.
38	F	2.2	80	+	Died within 1 hour.
6	F	1.72	100	+	
39	M	2.05	100	+	Died after 30 minutes.
7	F	1.72	150	+	Died after 5 minutes.

[1] Male=M; female=F. [2] Survived="–"; died="+."

Toxicity: M. L. D.=0.075 gm. per kilogram of body weight.

TABLE 11.—*Toxicity of novocaine hydrochloride in rabbits when administered subcutaneously.*

Rabbit No.	Sex.[1]	Weight in kilos.	Dose in mgs. per kilo body weight.	Result.[2]	Remarks.
29	M	2.64	100	–	
25	M	1.53	200	–	Died after 4 days from peritonitis.
13	M	2.1	257	–	
26	M	1.58	300	–	Died after 4 days. Cause unknown.
27	M	1.46	400	–	Died after 4 days from peritonitis.
23	F	1.6	400	+	Died after 20 minutes.
30	M	2.56	400	–	
24	M	1.6	500	–	
28	M	1.57	600	+	Dead after 12 minutes.
31	M	2.5	600	–	
32	M	2.1	700	+	Died after 12 minutes.
33	M	2.0	800	+	Do.

[1] Male=M; female=F. [2] Survived="–"; died="+."

Toxicity: M. L. D.=0.400 gms. per kilo of body weight.

In every case the novocaine was given in 20 per cent solution. The symptoms of novocaine and cocaine poisoning in rabbits closely resemble those which occur in guinea pigs.

The cocaine was given in either a 5 or a 10 per cent solution.

TABLE 12.—*Toxicity of cocaine hydrochloride in unanesthetized rabbits when administered intravenously (marginal ear vein).*

Rabbit No.	Sex.[1]	Weight in kilos.	Dose in mgs. per kilo body weight.	Concentration of injection fluid.	Result.[2]	Remarks.
				Per cent.		
4	M	2.1	5	1	–	Died after 21 days. Lung disease.
8	F	2.07	5	1	–	
16	M	2.2	7.7	2	+	Died after 3 minutes.
6	F	1.9	10	1	–	
15	M	2.9	10	2	–	
40	M	2.4	12	2	–	
17	M	2.4	15	2	+	Died after several minutes.
41	M	2.25	15	2	+	Do.
42	M	2.04	20	2	+	Do.
43	M	1.75	25	2	+	Do.

[1] Male=M; female=F. [2] Survived="–"; died="+".

Toxicity: M. L. D.=7.7 mgs. per kilo of body weight.
S. L. D. (surely lethal dose)=15 mgs. per kilo of body weight.

TABLE 13.—*Toxicity of novocaine hydrochloride in unanesthetized rabbits when administered intravenously (marginal ear vein).*

Rabbit No.	Sex.[1]	Weight in kilos.	Dose in mgs. per kilo body weight.	Concentration of injection fluid.	Result.[2]	Remarks.
				Per cent.		
9	M	2.47	5	2	–	
10	F	2.0	10	2	–	
5	F	2.05	10	2	–	
11	F	2.4	15	2	–	
7	F	1.85	20	2	–	
19	M	1.7	25	5	–	
18	F	1.66	30	5	+	Died after 9 minutes.
20	M	2.55	30	5	–	Died after 11 days.
44	F	1.7	30	5	–	
45	F	1.7	35	5	–	
46	F	1.55	40	5	+	Died after few minutes.
47	M	1.55	50	5	+	Do.
48	M	1.55	60	5	+	Do.

[1] Male = M; female = F.

[2] Survived = "–"; died = "+".

Toxicity: M. L. D. = 30 mgs. per kilo of body weight.

TABLE 14.—*Comparative toxicity of cocaine and novocaine in frogs, mice, rats, guinea pigs, and rabbits.*[1]

Animal used.	Method of administration.	Cocaine, M. L. D., in gms. per kilo of body weight.	Novocaine, M. L. D. in gms. per kilo of body weight.	Ratio toxicity, cocaine to novocaine.
Frogs (R. pipiens)	Subcutaneously.	1.000	0.700	1.0 to 1.
Mice	do	.100	.550	5.5 to 1
Rats	do	.200	2.000	10 to 1
Guinea pigs	do	.060	.600	10 to 1
Rabbits	do	.075	.400	5.3 to 1
Do	Intravenously.	.0077	.030	3.9 to 1

[1] The M. L. D. being the amount per kilogram of body weight required to kill within 24 hours.

From the above table it is seen that the relative toxicity of cocaine and novocaine varies and depends upon the animal used in making the determination. For warm-blooded animals the toxicity of cocaine is from 4 to 10 times greater than novocaine; while for the cold-blooded animal, the frog, the toxicity of novocaine is slightly greater than cocaine. The frog is the least susceptible animal to cocaine, while the guinea pig is the most susceptible animal. In rabbits it requires a relatively smaller intravenous than subcutaneous dose to kill with novocaine than with cocaine. To novocaine the rabbit is the most susceptible animal, while the rat is the least susceptible.

Rabbits and guinea pigs may be considered to be the most satisfactory test animals for determining the toxicity of these substances.

If cocaine or novocaine is given to anesthetized rabbits, intravenously, in dilute solutions at about five-minute intervals they will tolerate larger amounts of the drugs than when they are given in a single dose, as was done in the unanesthetized animals.

For example, a rabbit weighing 1.8 kilos, which was anesthetized with paraldehyde (1.7 c. c. per kilo by stomach) received during the course of one and one-half hours 49 mgs. of cocaine hydrochloride, intravenously, the equivalent of 27 mgs. per kilo of body weight. The drug was injected into the femoral vein in 2 to 8 mg. doses at intervals of about five minutes, each dose being dissolved in about 2 c. c. of distilled water.

To another rabbit weighing 1.64 kilos, which was similarly anesthetized, with the exception that a small amount of ether was occasionally required, 305 mgs. of novocaine were given before death resulted. This animal, therefore, received an amount of novocaine which was equivalent to 186 mgs. per kilo of body weight. The novocaine was injected into the femoral vein. It was given during the course of one and one-half hours in 5 to 20 mg. amounts, dissolved in 2 c. c. of distilled water.

When these experiments are considered, together with the results obtained in unanesthetized rabbits, we must conclude that when given slowly and in high dilutions an amount may be tolerated which is several times greater than that which is fatal when given as a single dose in concentrated solution. The concentration of the drug in the blood at any given time is a very important factor in determining the toxicity of either cocaine or novocaine. The rate of the injection, together with the concentration employed, must therefore be taken into account.

No extensive toxicological studies were made using the cat and dog as experimental animals. In order to find out the symptoms produced by novocaine in these animals a comparatively large dose was administered subcutaneously and its effects noted.

In a dog weighing 7.9 kilos a dose of 250 mgs. per kilo given subcutaneously, also in a cat weighing 2.7 kilos a dose of 500 mgs. per kilo given in the same manner produced symptoms of marked stimulation which lasted for about an hour only. From these two experiments it is seen that novocaine is somewhat similar to cocaine in that it possesses stimulating properties in these animals.

In feeding experiments with white mice the cocaine or novocaine was incorporated in cakes made up of cracker dust and milk and fed to white mice. The cakes were made in the following manner: For convenience, 128 gms. of fresh "soda" crackers, finely ground, were thoroughly mixed with a desired amount of the drug, previously dissolved in about 10 c. c. of water and 120 c. c. of milk. After mixing, the mass was then divided into 28 equal parts and dried in an open oven at 50° to 60° C. for about 24 hours. When dried each cake would then weigh about 4 gms. and would contain one twenty-eighth of the added drug.

Feeding experiments were conducted with white mice obtained from the laboratory stock.

The mice were kept in separate jars, food being withheld for 24 hours before the special feeding was begun. Each animal receive the drug in a 4-gm. cake of cracker dust and milk.

Control mice were kept in the same way, but were fed on all th plain cakes that they desired. This averaged a little less than on cake a day.

TABLE 15.—*Toxicity of cocaine hydrochloride when fed to white mice.*

Mouse No.	Sex.[1]	Weight in gms.	Amount cocaine eaten. in gms.	Result.[2]	Number of days lived.	Amount cocaine in cake in gms.	Remarks.
28	M	20.96	0.014	+	5½	0.005	
29	M	20.10	.011	+	6	.005	
30	F	17.34	.0075	+	9	.005	At autopsy the liver was pal grayish yellow and friable.
31	F	20.3	.0025	+	4	.010	
32	F	19.4	.00125	+	3	.010	
33	M	16.52	.00125	+	1½	.010	
34	M	20.5	.00125	+	1	.010	
35	F	18.65	.010	+	5½	.020	
36	M	19.25	.020	+	5	.020	
37	M	21.68	.020	+	5½	.020	
38	F	21.83	.0025	+	3	.020	

[1] Male = M; female = F.

[2] Survived = −; died = +.

Total weight of animals=216.53 gms.
Average weight of animals =19.68 gms.
Total amount of cocaine eaten=0.09125 gm.
Average amount of cocaine eaten by each mouse =0.0083 gm.
Average number of days mice lived=4.5 days.
Average lethal dose per gm. of body weight=0.000383 gm.
M. L. D.=0.383 gm. per kilo of body weight.

Upon autopsy, in all of these animals the liver was pale yellow i color. This change was especially pronounced in animal No. 30.

The mice in this series that were fed on cakes containing 0.0025 gm of cocaine hydrochloride lived.

TABLE 16.—*Toxicity of novocaine hydrochloride when fed to white mice.*

Mouse.	Sex.[1]	Weight in gms	Amount of novocaine eaten. in gm.	Result.[2]	Number of days lived.	Amount novocaine in cake in gm.	Remarks.
44	M	20.28	0.350	−	....	0.050	No effect; experiment discontinued after 8 days.
43	F	19.70	.350	−	....	.050	Do.
45	M	19.60	.350	−	....	.050	Do.
39	M	20.50	1.700	−	....	.100	No effect; experiment discontinued after 17 days.
40	M	18.35	.600	−	....	.100	No effect; experiment discontinued after 7 days.
72	F	22.13	.800	−	....	.200	No effect ;experiment discontinued after 6 days.
78	M	23.66	.400	+	5	.400	Liver congested very dar brown color.
79	M	23.80	.800	+	6	.400	Do.

[1] Male = M; female = F.

[2] Survived = −; died = +.

From the above and the preceding table it will be seen that novocaine is relatively much less toxic than cocaine when fed to mice. I we consider 0.400 gm. as the minimum lethal dose by mouth, the relative toxicity of cocaine and novocaine in this series would be about 50 to 1.

The animals would readily eat the cakes containing 0.050 to 0.100 gm. of novocaine, whereas cakes containing larger amounts would be refused.

For control purposes five mice from the laboratory stock were fed on plain cakes and otherwise kept under the same conditions as the mice which received the cocaine and novocaine. All of them lived and gained in weight.

Other experiments were conducted, with mice from the laboratory stock, to determine whether feeding novocaine in sublethal doses for a period of weeks would confer a certain immunity to cocaine when given in the same way. The experiments were not satisfactory. It appeared, however, that the mice which received novocaine in this way reacted to cocaine to the same degree as normal mice.

The symptoms produced in mice by cocaine, when fed in an amount which would ordinarily be fatal in about two days, resemble those which are produced by cocaine in these animals when administered subcutaneously.

The following experiment shows the effect of feeding cocaine to mice:

Mouse No. 9, male; weight, 14.23 gm.:

10.00 a. m., fed a 4 gm. cracker-dust and milk cake containing 0.010 gm. cocaine. Mouse ate sparingly throughout the day. No symptoms.

Next day, 8 a. m., mouse had eaten 2.5 gm. of the cake. Animal was restless. *Some rigidity in hind legs.*

9 a. m., very drowsy; respiration irregular and slow.

10.30, dyspneic; will jump at slightest touch.

10.45, spontaneous abortive clonic convulsion.

11.00, lying on side; reflexes increased.

11.05, respiration practically stopped; gasps about eight times per minute.

11.19, tonic convulsion; remains rigid for about one minute with back humped and legs extended.

11.21, dead, body rigid.

The mice which were fed on novocaine showed few symptoms. Shortly before death they became quiet and very weak in their hind legs. Some showed muscular tremors.

PART II.

ACTION ON HEART MUSCLE.

The effect of cocaine and novocaine on heart muscle was studied on the isolated heart of the frog, using both the *Rana esculenta* and the *Rana pipiens*. Perfusion was done with the drugs dissolved in Clarke's solution.[1]

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[1] Clarke's solution was made up as follows: $NaCl$ 0.7 per cent; $CaCl_2$ 0.012 per cent; KCl 0.014 per cent; $NaHCO_3$ 0.02 per cent.

When the *Rana esculenta* was used the perfusion was done through the aorta according to the method of Straub, 1901. In this method a glass cannula having a capacity of about 1 c. c. was introduced into the ventricle through the aorta. After tying in the cannula, a thin wire, which is preferably made of platinum, was introduced through the tip of the ventricle for the purpose of recording the movements of the ventricle on a kymograph.

The perfusion of the isolated heart of the *Rana pipiens* was done through the vena cava. An inflow cannula was introduced into one of the anterior venæ cavæ and an outflow cannula into the aorta. The cannulæ were then tied and the remaining vessels ligated. A record of the movements of the ventricle was obtained in the same way as in the Straub method.

The Straub method was found to be better adapted for use with the heart of the *Rana esculenta* than for the heart of the *Rana pipiens;* an especial advantage which this method possessed is its adaptability to small amounts of fluid.

EFFECT OF COCAINE AND NOVOCAINE ON ISOLATED HEART OF RANA ESCULENTA.

Cocaine (*Straub method*).—The effect of cocaine on the isolated heart of *Rana esculenta* depended upon the concentration employed Weak concentrations (1 to 10,000) usually produced a marked decrease in systole, together with a slight decrease in diastole. (Fig. 1.) There was usually a varying increase in rate. In certain experiment the increase in the heart rate was only several beats per minute whereas in other experiments the increase in rate was quite marked being as great as 10 beats per minute. In still other experiment there was a decrease in rate. When the concentration 1 to 5,000 wa employed, a decrease of several beats per minute occurred, togethe with a marked decrease in systole and a slight decrease in diastole Recovery was prompt when concentrations of 1 to 10,000 and 1 t 5,000 were employed. Dilutions as concentrated as 1 to 100 woul produce a very prompt decrease in systole and finally stoppage i diastole. Recovery from this dilution was generally prolonged a compared with the recovery from higher dilutions.

Novocaine (*Straub method*).—By the Straub method novocain in dilutions of 1 to 10,000 to 1 to 5,000 caused, in the isolated hear of *Rana esculenta*, an increase in rate similar to that caused by co caine. Unlike cocaine, there was little or no effect on the amplitud of the contraction when the higher dilution was used; whereas whe the 1 to 5,000 dilution was employed there was a slight decrease i the extent of systole. Figure 1 shows an effect not usually obtaine with 1 to 10,000 dilutions, namely, a decrease in the extent of systoli contraction followed by a slight increase in the amplitude of systolic contraction shortly after the drug was administered.

EFFECT OF COCAINE AND NOVOCAINE ON ISOLATED HEART OF RANA PIPIENS.

Cocaine (perfusion through vena cava).—When the isolated heart of *Rana pipiens* was perfused through the vena cava with cocaine in very high dilutions, 1 to 160,000 and 1 to 100,000, there was produced either no change or a slight decrease in rate. With these dilutions there was always a decrease in the extent of systole. A more concentrated dilution, 1 to 20,000, would cause a rapid decrease in rate together with a decrease in the extent of systole, so that after ½ to 2 minutes the heart stopped in diastole. (Fig. 2.)

The effect of cocaine on the isolated heart of *Rana pipiens* is, therefore, unlike the effect on the heart of the *Rana esculenta*, since with *Rana pipiens*, in no case was an increase in rate observed when higher dilutions were used, as was observed in the *Rana esculenta.*

An effort was made to prevent or abolish the slowing produced by weak dilutions of cocaine, by applying atropine to the heart, either before slowing had occurred or after it was present; but in no instance did it have any effect on the slowing produced by cocaine. The cause of the slowing produced by cocaine in the frog, therefore, is due to a direct effect upon the heart muscle.

The effects of cocaine on the heart of *Rana pipiens* which are described above agree with those obtained by von Anrep (1879) on the intact heart of *Rana esculenta.* Von Anrep found that, in *Rana esculenta*, doses of 0.005 to 0.0015 gm. of cocaine, applied to the exposed heart *in situ*, produced no change in the extent of contraction nor in the rate; and that doses of 0.003 gm. promptly caused a decrease in rate and in the extent of contraction, finally producing diastolic standstill. These results agree with the findings of Kuroda, 1915, on the isolated heart of *Rana temporaria.* They do not agree with the results obtained by Mosso, 1887, who noted in *Rana esculenta* not only an increase in rate but in the extent of the contraction as well.

Novocaine (perfusion through vena cava).—The effects of novocaine on the isolated heart of *Rana pipiens*, when perfused through the vena cava are very similar to the effects produced by cocaine, namely, a decrease in rate accompanied by a decrease in the extent of systole, when either dilute or concentrated solutions were used. (Fig. 2.) As with cocaine, the heart is very quickly stopped in diastole by concentrated solutions. In one instance atropine partly removed the slowing caused by a 1 to 20,000 dilution of novocaine, but the result could not be obtained in other experiments. This would indicate, however, that, in addition to having an effect on heart muscle, it may stimulate the vagus endings in the frog's heart.

The relative toxicity on the heart as shown by perfusion of the frog's heart was about 1 to 4, i. e., it required 4 times more novocaine than cocaine to produce a similar depression.

PART III.

ACTION ON SMOOTH MUSCLE.

The effects of cocaine and novocaine on smooth muscles were compared by observing the response of isolated segments of the ureter of the dog, intestine of the rabbit, urinary bladder and stomach of the cat, and uterus of the rabbit when placed in varying dilutions of the drugs in Ringer's solution.

In studying the effects of cocaine and novocaine upon the ureter, the ureter of the dog was selected, as it was found to give spontaneous rhythmical contractions when placed in Locke's solution and was sensitive to both drugs. The ureters of the rabbit and of the cat were found to be inactive when placed in Locke's solution, both as regards spontaneous rhythmical contractions and reaction to cocaine and novocaine under the conditions of the experiment. Some of the dogs which were used were either etherized or narcotized with chloretone before removing the ureteral segment, while others were killed either with illuminating gas or painlessly by mechanical means and quickly bled before taking the ureteral segment for the experiment. As a rule, 2 to 3 inches of either ureter were taken near the middle third and mounted in the ordinary way in Locke's solution, warmed to 38° C., the segment being weighted with from 0.5 to 0.7 gm. weights. Large dogs were selected on account of the size of the ureter. Regardless of how the animal was prepared before obtaining the segment, the rhythmical contractions which may occur after it is placed in Locke's solution and weighted as a rule do not occur before one hour. The ureteral segment should be well oxygenated with a constant supply of oxygen. The ureter of the dog usually showed rhythmical contractions, which appeared at the rate of one every two or three minutes to one to three each minute. Occasionally spontaneous rhythmical contractions did not appear until after cocaine or novocaine was used, the contractions then being at fairly regular intervals. Two kinds of contractions were noticed in some segments. Ordinarily there was a single marked contraction every two to three minutes or a single contraction each minute. At times, however, with certain segments two or three small contractions appeared between the large contractions.

EFFECT OF COCAINE AND NOVOCAINE ON ISOLATED URETER OF DOG.

Cocaine.—The effect of cocaine on the isolated ureter of the dog, when arranged in the above-described manner, is as follows:

With a dilution of 1 to 100,000 an increase in height and in the number of the spontaneous contractions occurs several minutes after immersion in the solution. (Fig. 3.) The height of the contractions may not be affected, the only effect in certain cases being a slight increase in the number of contractions. Occasionally, in addition to the increase in the height and number of contractions, there is a slight increase in tonus. With a more concentrated solution (1 to 40,000) an increase in number and height of contractions always occurs, sometimes accompanied with an increase in tonus. If the solution is still more concentrated (1 to 20,000 or 1 to 10,000), a marked increase in the number of contractions occurs, together with considerable increase in tonus, the tonus at times with the 1 to 10,000 dilution causing on certain segments incomplete tetanus. Complete tetanus is caused with 1 to 5,000 dilutions after several minutes, from which recovery occurs in 10 to 15 minutes after replacing the segment in Locke's solution. (Fig. 4.)

Novocaine.—The effect of novocaine on the ureter is qualitatively similar to that of cocaine.

The isolated ureter of the dog reacts to weak dilutions of novocaine in precisely the same degree as to cocaine, but when more concentrated dilutions are used the effects while qualitatively similar are quantitatively dissimilar. A 1 to 100,000 dilution will cause an increase in the height and number of the spontaneous rhythmical contractions (Fig. 3), and in certain segments a slight increase in tonus occurs; while for the production of tetanus a more concentrated solution of novocaine is required than of cocaine, the dilution necessary to produce this effect being 1 to 2,500. (Fig. 5.) Taking the production of this phenomenon as a criterion, cocaine is about five times as active as novocaine.

EFFECT OF COCAINE AND NOVOCAINE ON ISOLATED INTESTINE OF RABBIT.

Cocaine.—In the experiments on the isolated intestine, the intestinal preparation was secured from rabbits which had been anæsthetized with 1.5 gm. urethane per kilo by stomach. The segment was usually about 1 to 1.5 inches long, and was taken from the lower part of the small intestine. The recording of its movements was done in the usual way.

The action of cocaine on the isolated intestine of the rabbit differs essentially from that of novocaine. Dilutions varying from 1 to 250,000 to 1 to 5,000 were tried. With the 1 to 250,000 dilution there was no change in rhythm, but usually a marked gradual increase in tonus and at all times an increase in the amplitude of the contraction. (Fig. 6.) When 1 to 20,000 dilutions were used, there was a moderate decrease in the rhythm of the spontaneous contractions and in the amplitude of the contractions, without special change in the tonus. Late in the experiment, irregularities in the contractions appeared. Increasing the concentration to 1 to 5,000 caused a prompt partial cessation of the spontaneous movements. (Fig. 7.)

In some segments the tonus was maintained for some time, although the spontaneous rhythmical contractions were irregular and infrequent. If allowed to continue longer in the solution the tonus decreased.

Novocaine.—Novocaine, in as weak a dilution as 1 to 400,000, produced a slight decrease in tonus and in the extent of the amplitude of the contraction but no change in rhythm. This was true when dilutions varying from 1 to 400,000 to 1 to 20,000 were employed. (Fig. 6.) When either 1 to 20,000 or 1 to 10,000 dilutions were used little change in rhythm was produced. Sometimes a very slight decrease in rate of the rhythmical contractions followed. In addition, there was a slight loss of tonus and considerable decrease in the extent of the rhythmical contractions. A 1 to 5,000 dilution produced an effect similar in most respects to that caused by a like dilution of cocaine; namely, a decrease in the rate and extent of the amplitude of the contraction, together with irregularities in the extent of the contraction. (Fig. 8.)

EFFECT OF COCAINE AND NOVOCAINE ON ISOLATED URINARY BLADDER OF CAT.

Before removal of the bladder the cat received 1 gm. of urethane per kilo by stomach. Median sagittal segments of the bladder were used which, when relaxed with about 1 to 1.5 gm. weights, measured about 3 inches in length. The segment was mounted and its movements were recorded in the usual way.

The effects of cocaine and novocaine on segments of the urinary bladder of the cat were practically alike. Dilutions of 1 to 5,000 and 1 to 100,000, of both cocaine and novocaine, were tried and in these dilutions the segments responded by contracting. There was very little, if any, quantitative difference in the response; the cocaine effect was a trifle greater than that of the novocaine. In certain experiments, the spontaneous movements were increased in number and height by both drugs, and in other experiments they were uninfluenced. The effects are illustrated in Fig. 9.

EFFECT OF COCAINE AND NOVOCAINE ON ISOLATED STOMACH OF CAT.

For the determination of the effect of cocaine and novocaine on the isolated stomach, a strip of stomach wall taken from the pyloric end was used. These strips were taken from the animals in the same manner as was described in the experiments on the urinary bladder, and the effects similarly recorded. Both cocaine and novocaine, in dilutions varying from 1 in 20,000 to 1 in 2,500, were used and in all experiments the effects were qualitatively the same, namely, an increase in tone or a distinct contraction, together with an increase in the amplitude of the spontaneous movements. The cocaine action was a trifle more pronounced than was the novocaine action. (Fig. 10.)

EFFECT OF COCAINE AND NOVOCAINE ON ISOLATED UTERUS OF RABBIT.

The effects of cocaine and novocaine were ascertained in the usual way on uterine segments, taken from either nonpregnant or pregnant animals which were narcotized with urethane. (1.5 gms. per kilo).

Cocaine and novocaine caused contraction in segments taken from both nonpregnant and pregnant animals, the latter being more sensitive to both cocaine and novocaine than the former. The dilutions used were 1 to 100,000 and 1 to 10,000. (Fig. 11.)

PART IV.

EFFECTS OF COCAINE AND NOVOCAINE ON BLOOD PRESSURE AND RESPIRATION.

EFFECT ON BLOOD PRESSURE.

The blood-pressure changes resulting from cocaine and novocaine were studied in dogs, cats, and rabbits. In each species the effects in anesthetized animals were recorded graphically by obtaining a record of the blood-pressure fluctuations which occurred in the carotid artery after the intravenous administration of known amounts of the drugs. The drugs were, as a rule, slowly injected into the femoral vein but occasionally in rabbits the jugular vein was used. As anesthetics for rabbits, ether or paraldehyde (1.7 c. c. per kilogram by stomach) was employed; while for cats and dogs, either chlorbutanol (0.3–0.4 gm. per kilo by stomach) or ether was used.

The effects of cocaine and novocaine on the blood pressure of dogs, cats, and rabbits are essentially alike. When either drug is given intravenously in small doses to anesthetized animals a rise in pressure usually occurs while after large doses a fall in pressure is produced. (Figs. 12 to 20, inclusive). As an exception to the above findings, there may also be a secondary fall in pressure following the rise obtained by the use of small doses. This secondary fall is inconstant. The rise which occurs after the use of small doses of cocaine is generally higher than the rise produced by novocaine, and it is produced by smaller doses.

The dose of novocaine, required to produce a rise in pressure in the rabbit, varies from 5 to 15 mg., while in the dog 10 to 40 mg.

are required. The rise may be seen in cats after doses varying from 5 to 20 mg. In dogs and rabbits the rise is not always seen; in some rabbits 5 mg. may produce a fall. By the use of doses larger than 20 mg. a fall usually occurs in either dogs, cats, or rabbits, although in some dogs 40 mg. will produce a rise. A dose which produces a rise in pressure on first injection may, if given later in the experiment, produce a fall in pressure.

The effect of novocaine on the blood pressure of the rabbit is shown in the following abstract from an experiment:

EXPERIMENT 1.—*Rabbit.*

Female, weight, 1.64 kilos.; anesthetized with paraldehyde; carotid blood pressure; drug injected into femoral vein; vagi intact; light anesthesia.

Time.	Drug injected.	Blood pressure fluctuations.	Remarks.
1.52	5 mg. novocaine	90 to 100 mm. Hg	Rise of 10 mm. Hg.
2.00	10 mg. novocaine	90 to 70 mm. Hg	Fall of 20 mm. Hg.
2.25	20 mg. novocaine	90 to 50 mm. Hg	Fall of 40 mm. Hg.

There was no effect on the respiration in this experiment from 5 to 10 mg. doses, but with the 20-mg. dose respiration became shallow and slower.

In the next experiment the effect of cocaine on the blood pressure of the rabbit is shown.

EXPERIMENT 2.—*Rabbit.*

Male, weight 1.84 kilos, anesthetized with paraldehyde, carotid blood pressure, drug injected into the femoral vein, vagi intact, anesthesia moderately profound.

Time.	Drug injected.	Blood pressure fluctuations.	Remarks.
10.43	2 mg. cocaine	66 to 100 mm. Hg	Rise of 34 mm. Hg.
11.00	4 mg. cocaine	64 to 48 to 100 mm. Hg	Fall of 16 mm. Hg. followed by rise of 36 mm. Hg.
11.50	do	55 to 38 mm. Hg	Fall of 16 mm. Hg.; no secondary rise.

At 10.46 the respiration was much deeper but was slowed from 96 to 83 per minute.

In the following protocol the effect of cocaine and novocaine on the dog are shown in the same experiment.

EXPERIMENT 3.—*Dog.*

Male, weight 7.6 kilos, ether, carotid blood pressure, drugs in femoral vein, vagi intact.

Time.	Drug injected.	Blood pressure fluctuations in mm. of Hg.	Remarks.
10.19	40 mg. cocaine	140-148-122	Rise of 8 mm., and then fall of 18.
10.52	40 mg. novocaine	140-150-106	Rise of 10 mm., then fall of 34.
11.25	40 mg. cocaine	146-108	Fall of 38 mm.
1.37	40 mg. novocaine	122-94	Fall of 28 mm.

EFFECT ON RESPIRATION WHEN GIVEN INTRAVENOUSLY.

The effects of cocaine and novocaine on the respiration were studied in dogs, cats, and rabbits. The animals were anesthetized with ether, paraldehyde, or chlorbutanol in the manner and dosage previously described. In order to record graphically respiratory changes, two methods were employed. In the first method a metal cannula was inserted into the pleural cavity through a small opening in the chest wall and connected with a tambour, the changes being recorded on a moving surface. This method, therefore, gave a record of volume changes in the entire chest cavity. In the other method a sensitive tambour was placed upon the chest wall near the diaphragm and was then connected to a double-lever tambour, which recorded its movements on a revolving drum. In the latter method the movements were for the most part diaphragmatic and gave no accurate indication as to the volume changes in the chest cavity. This method will be referred to as the "tambour method" and the former as the "pleural cannula method." When studied by the above-described methods it was found that novocaine resembles cocaine in its effect upon the respiration of dogs and rabbits, cocaine markedly stimulating the respiration when small doses were used and depressing it when larger doses were employed; whereas novocaine as a rule had a slight stimulating effect in small doses and in larger doses depressed respiration. (Figs. 12 to 20, inclusive.)

In the cat a stimulating effect on the respiration was never noticed; however, the experiments in which small doses were used were few, and in those experiments even 5 mg. doses caused a decrease in respiration. (Fig. 20.) By the pleural cannula method in one dog a 5 mg. dose of novocaine caused an increase in the rate and depth of respiration without producing any effect on the blood pressure. (Fig. 16.) In other dogs 5–10 mg. doses of novocaine would increase the depth of respiration but leave the rate unchanged. A dose of 25 mg. of either cocaine or novocaine usually decreased both the rate and amplitude of the respiratory movements.

EFFECT OF NOVOCAINE ON BLOOD PRESSURE AND RESPIRATION OF RABBIT WHEN GIVEN SUBCUTANEOUSLY.

In order to find out the effect of novocaine on the blood pressure and respiration when a large dose is injected subcutaneously, a 2-kilo rabbit, anesthetized with paraldehyde, was given a subcutaneous injection of 0.2 gm. of novocaine dissolved in 4 c. c. of water. The blood pressure was recorded in the usual way and the respiration was ascertained by means of the pleural cannula method. The changes are shown in the following abstract from an experiment:

Time.	Respiration per ½ minute.	Amplitude of respiration in mm.	Heart rate per ½ minute.	Blood pressure in mm. of Hg.
10.39	50	7	108	74
11.29	44	5	95	52

There is thus seen to occur in the rabbit a distinct depression of respiration and blood pressure and a decrease in heart rate when given in the above manner.

COMPARATIVE EFFECTS OF COCAINE AND NOVOCAINE ON BLOOD PRESSURE AND RESPIRATION OF DOGS WHEN GIVEN SUBDURALLY.

An effort was made to determine the relative toxicity of novocaine and cocaine when given intraspinally. Dogs were selected, in preference to rabbits or cats, on account of the fact that the subdural space in the dog is comparatively large and because the dura may be lifted without apparent injury to it. A comparison of the blood pressure and respiratory effects resulting from comparatively large doses of cocaine and novocaine was made in etherized dogs. The effect on the blood pressure was recorded in the usual way and the effect on the respiration was obtained by the pleural cannula method. In order to inject the drugs subdurally, the second and third lumbar vertebræ were partially removed, and the dura exposed.

Experiment 4.—*Dog.*

(Weight, 12 kg. Etherized.)

Time.	Drug injected.	Blood-pressure fluctuations.	Remarks.
12.25	40 mg. cocaine	138-76 mm. Hg	Fall of 62 mm. Hg.
1.41	40 mg. novocaine	140-82 mm. Hg	Fall of 58 mm. Hg.

EFFECTS ON RESPIRATION.

Time.	Drug injected.	Respiratory changes			
		Amplitude.		Number in 10 seconds.	
		Before.	During.	Before.	During.
12.25	40 mg. cocaine	6	4	13	9
1.41	40 mg. novocaine	7	9	15	3

Experiment 5.—*Dog.*

(Weight, 9 kg. Etherized.)

EFFECTS ON THE BLOOD PRESSURE.

Time.	Drug injected.	Blood-pressure fluctuations.	Remarks.
1.27	40 mg. novocaine	132–60 mm. Hg	Fall of 72 mm. Hg.
3.21	40 mg. cocaine	116–50 mm. Hg	Fall of 66 mm. Hg.

EFFECTS ON THE RESPIRATION.

Time.	Drug injected.	Respiratory changes.			
		Amplitude.		Number in 10 seconds.	
		Before.	During.	Before.	During.
1.27	40 mg. novocaine	5	5	3.5	2
3.21	40 mg. cocaine	12	18 3	4.5	17 2

From the above experiments it is seen that a fall in pressure results from the use of either cocaine or novocaine in large doses, and the extent of fall is practically the same for both substances. The respiratory effects are unlike, however, in that cocaine may stimulate respiration previous to depressing it; whereas with novocaine there is little or no primary stimulation preceding the depression. These experiments would indicate that there is little, if any, difference in the toxicity of novocaine and cocaine when given intraspinally to dogs.

No systematic study was made to determine the point of action of novocaine which would explain the reason for its effects on the circulation and respiration. In a few animals, however, the heart effects were noted simultaneously with the changes in the blood pressure. In these experiments the heart was exposed, and a record of the movements of the left ventricle was obtained by means of the Cushny myocardiograph; at the same time a record of the blood-pressure changes was obtained from the carotid artery.

In the intact heart of the cat there is a distinct increase in the systole of the left ventricle with from 5 to 20 mg. doses of novocaine (Fig. 21), which is coincident with the rise in pressure; whereas after 80 mg. doses of novocaine in the cat, there is a decrease in the contraction of the left ventricle, which is also coincident with the fall in blood pressure. (Fig. 22.) These phenomena occur regardless of whether the vagi are intact or cut.

In the dog a 5 mg. dose of novocaine caused a slight but distinct increase in the contraction of the left ventricle, but at the same time it caused a fall in the blood pressure (fig. 23), while larger doses (0.040 gm.) caused a decrease in the systole of the left ventricle, together with a fall in the blood pressure. (Fig. 24.)

Stimulation was not noticed in the intact rabbit's heart when determined in the above way, the effect always being either a negative or a depressant one.

The heart rate was always markedly slowed after large doses of novocaine in every species of animals used.

CAUSE OF DEATH FROM COCAINE AND NOVOCAINE POISONING.

The cause of death from novocaine poisoning was investigated on dogs and rabbits. In dogs which had received novocaine either intravenously or intraspinally the respiration stopped from about one-half to one minute before the heart. In rabbits death would always occur from respiratory failure if the novocaine was injected slowly into the marginal ear vein. In these instances the animal would live for some minutes after the injection, as, for instance, when the drug was injected intravenously and the injection period was 2 to 3 minutes long. If the novocaine was injected rapidly, the animal receiving the entire amount in from one-fourth to one-half minute, death would be cardiac, the heart stopping under these conditions 10 to 15 minutes before the respiration had ceased. Death from novocaine is usually respiratory, therefore, but under certain conditions it may be cardiac, the interval between the stoppage of the heart and respiration being very short. In both rabbits and dogs death from cocaine was always respiratory, regardless of the method of its injection.

PART V.

GENERAL CONSIDERATIONS.

TOXICITY OF COCAINE AND NOVOCAINE IN MAN.

It is impossible to determine accurately the relative susceptibility of man to cocaine and novocaine, on account of the fact that the cases of novocaine poisoning are just beginning to find their way into the literature. However, from physiological tests on man and from the available reports of the cases of poisoning an approximate idea of the relative toxicity in man may be computed. Blythe, 1906, states that according to Mannheim, 1 gm. of cocaine must be considered the fatal dose for man. Blythe further states that the smallest dose known to have been fatal for an adult is 0.08 gm. and 0.05 gm. for a child.

Witthaus and Becker, 1911, compared critically the reports of 384 cases of cocaine poisoning previous to 1909. Practically all of the cases in their series, prior to 1894, were accidental cases which resulted from its use in surgical practice. Of the fatal cases two were children, aged 2½ and 11 years, respectively, who received 0.032 gm. (per urethra) and 0.024 gm. (intramuscularly; deltoid), respectively. The smallest lethal dose of cocaine by mouth for adults is given as 0.648 gm., while in two cases of nonhabitués 1 gm. and 1.1 gm. did not produce death.

Falk, 1890, tabulated 176 cases of cocaine poisoning, 10 of which were fatal. Among these cases is that reported by Abadie of an aged woman in whom 0.040 gm. of cocaine was fatal when injected into the subconjunctival tissues. In Falk's series is a fatal case following the use of 0.225 gm. of cocaine when injected subcutaneously into the breast.

Ricci, 1887, reported recovery from a dose of 1.25 gm. of cocaine, when injected subcutaneously into the leg tissues.

Mattison, 1891, states that the smallest fatal subcutaneous dose of cocaine reported in the literature is 8 drops of a 4 per cent solution (probably 0.020–0.030 gm.).

Toxic symptoms from cocaine begin to appear when very small amounts are given. Minor, 1885, collected cases in which very small doses caused toxic symptoms. In one of these cases reported by Stevens, 4 minims of a 3½ per cent solution of cocaine (0.008 gm.) injected into the subcutaneous tissues of the orbit, caused within 10 minutes difficult breathing, convulsions, and unconsciousness.

In summarizing the cocaine cases, we find that the fatal dose varies between 0.020–0.030 gm. to more than 1.25 gm., while 0.008 gm. may produce dangerous toxic symptoms. Cushny, 1915, states that, for subcutaneous injection, the dose of cocaine should never exceed 0.020 gm.

The maximum dose of novocaine tolerated by man without producing general symptoms, like other local anesthetics, depends largely upon the method of its administration. The dosage used by clinicians is remarkably wide.

Danielsen, 1905, never noticed toxic symptoms from amounts up to 0.100 gm., when injected subcutaneously, if given in 1 to 2 per cent solution.

Schmidt, 1905, used doses as large as 0.250 gm., in 1 per cent solution subcutaneously, without observing toxic effects.

Nast-Kolb, 1908, uses as much as 50 c. c. of a 1 per cent solution (0.5 gm.) subcutaneously.

Eastman, Erdman, and Bonn, 1916, state that for some operations 1 to 1.6 gm. are employed.

Braun, 1914, states that Axhausen used as much as 2 gm. subcutaneously without producing harmful effects. Braun has used in the same way as much as 250 c. c. of a 1 per cent solution (1.25

gm.) without producing any secondary effects, except occasionally vomiting.

Kreche, 1910, by mistake, gave 2 c. c. of a 20 per cent solution (0.4 gm.) of novocaine subcutaneously in an operation for carcinoma of the larynx and observed no general effects from it whatever.

Liebl, 1906, considers 0.4 gm. as a safe dose for an adult and states that doses as high as 0.75 gm. may give rise to general symptoms when used as a local anesthetic. Liebl injected 1.15 gm. into himself subcutaneously in two doses. The first dose of 0.4 gm. was injected subcutaneously into his thigh as a 10 per cent solution and caused no toxic symptoms. About 1 hour later the second dose of 0.75 gm. was injected into the subcutaneous tissues of the forearm causing within 5 minutes a feeling of nausea and some uneasiness. About 15 minutes later there was inability to concentrate, headache, double vision, lack of accommodation and deafness, together with paraesthesia along the course of the radial nerve. Within 1 hour after the second dose these symptoms had disappeared almost entirely.

Schlesinger, 1912, injected 15 c. c. of a 2 per cent solution of novocaine (0.3 gm.) into the brachial plexus of a man with pronounced arteriosclerosis and observed slow pulse, generalized twitchings of the body and collapse. These phenomena passed off in several minutes. Since this experience he has used smaller doses.

Begg (1913) reported the case of a man who received 1 grain (0.065 gm.) of novocaine combined with about three-fourths of a minim of a 1 to 1,000 solution of epinephrine subcutaneously in the tissues of the chin. Without the slightest warning the pupils became widely dilated and fixed, unconsciousness ensued, and a cold, clammy sweat broke out upon his face. Both respiration and heart were slow, and the whole body became rigid. Recovery occurred within one hour.

Landsberger (cited by Kehr, 1910) observed severe headache, paleness, general malaise, and numbness of the fingers in a girl who had received subcutaneously a small dose of a 2 per cent solution of novocaine combined with suprarenin.

Fischer and Riethmüller (1914) observed narcotic slumber following the injection of 0.66 gm. of novocaine for the extraction of teeth. Although the novocaine was given with thymol and suprarenin, in their opinion the narcosis was due to the novocaine and the unusual susceptibility of the patient.

Giffin and Gundrum (1914) reported that general symptoms resulted from one-third grain (0.020 gm.) of novocaine combined with one two-hundredth grain of synthetic suprarenin when injected into the peridental mucous membrane. Another case of nonfatal poisoning was reported to them as occurring after one-sixth grain (0.010 gm.) when injected in the same way.

Kehr (1910) gave one-half of a syringeful of a one-half per cent solution of novocaine (probably less than 0.025 gm.) to secure anesthesia for the extraction of teeth and produced pulse changes, paleness, headache, chills, dilatation of the pupils, respiratory disturbances, convulsive movements, and unconsciousness. The patient was revived by employing artificial respiration, and after several hours recovery was complete.

Marshall (1914), in relating his personal experience with novocaine, says that he was given during an hour three injections, or a total of 0.090 gm., of novocaine, combined with adrenalin, for the removal of teeth, and after the second and third injections he experienced a slight increase in pulse rate, accompanied by deep and rapid respiration. These symptoms lasted for three or four minutes only. That night he complained of headache and chilly feet, and passed a restless night. The next day while out walking he was seized with a feeling of constriction in the region of his heart, and his breathing became labored. His pulse increased from 68 to 75. After a short rest the symptoms disappeared.

For spinal anesthesia, Page (1915) gives 2 to 2.5 c. c. of a 5 per cent solution (0.100–0.125 gm.). In the same way Holman (1916) uses 0.060–0.080 gm. in 5 per cent solution. Holman cites two nonfatal cases of novocaine poisoning. In both cases 0.080 gm. of novocaine had been given intraspinally.

Heineke and Läwen (1905) also consider the dosage of novocaine for spinal anesthesia to be 0.1 to 0.15 gm. when given as a 5 to 10 per cent aqueous solution. They have given as much as 0.18 gm. in this way. In two cases in which 0.15 gm. was given intraspinally marked weakness of the pulse and pallor, together with nausea and vomiting, followed its administration.

Hofmann, 1906, states that 2 to 3 c. c. of a 5 per cent solution (0.1 to 0.15 gm.) of novocaine is usually employed for spinal anesthesia. He, however, is inclined to use smaller doses (0.050 to 0.070 gm.). Even with these doses he has occasionally noticed headache and vomiting as accompanying symptoms. A colleague of his observed malaise followed by a feeling of well being, from 5.5 c. c. of a 1 per cent solution, when given subcutaneously for a hernia operation.

Gabbett, 1910, administered intraspinally 3 c. c. of a solution containing 0.100 gm. of novocaine and 0.001 gm. of strychnine. Ten minutes after the injection the patient had difficulty in breathing, the body became rigid and death occurred, without convulsions.

Fullerton, 1913, considers that novocaine was the cause of death in a man, aged 68, who had a weak heart and a double aortic murmur, to whom 1 c. c. of a 15 per cent solution of novocaine (0.150 gm.)

with 0.001 gm. of strychnine was given intraspinally. Soon after the injection the patient began to vomit, and death ensued several hours after the operation.

Braun, 1914, mentions two fatal cases reported by Claus. In one case a tampon containing 6 drops of a 10 per cent solution (0.040 gm.) of novocaine and 6 drops of adrenalin was placed in the nose for 20 minutes. After removal of the tampon the antrum was washed. Almost immediately after this procedure the patient became cyanotic and died of cardiac paralysis. In the other case, a tampon containing novocaine and suprarenin was used in the nasal tract and in addition a 10 per cent cocaine solution was applied locally to the mucous membrane of the nose. Following the local anesthesia the antrum was punctured and inflated; the patient collapsed and died the same evening. Inasmuch as Claus also reported two serious cases in which a similar operation was performed without anesthesia, one of which was fatal, Braun does not regard the cases reported by Claus as cases of novocaine poisoning.

Levy and Hatcher, 1915, reported the death of a patient who had received a small dose of novocaine combined with synthetic suprarenin.[1]

Scandola, 1915, reported a case of death which resulted from the intraspinal administration of a solution made from two tablets, each said to contain 0.050 gm. of novocaine and 0.000083 gm. of suprarenin. He also states that his colleague, Merusel, has observed serious symptoms, even with small doses of novocaine, and in two instances death occurred. He considered novocaine as the cause of death in both cases.

Michelsson, 1912, collected from the literature 13 fatal cases of novocaine poisoning which resulted after its use intraspinally. Four of these cases were reported by Füster, who did not regard novocaine as the cause of death in any of them. The remaining 9 cases were those reported by Sonnenburg (1), Bosses (1), Mohrmann (1), Silberberg (1), Konig (1), Jolly (1), Risch (2), and Waitz (1). The dose of novocaine varied in these cases between 0.050 to 0.200 gm.

From the above references it is seen that no definite conclusion can be drawn as to the exact toxicity of novocaine in man. Under certain conditions 0.020 gm. has caused poisoning symptoms; whereas 2 gms. have been given in other cases without producing symptoms. It would appear, however, that when given intraspinally or for dental operations it may be especially toxic. By the intraspinal method of administration 0.100 gram has produced death.

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[1] Since the above bibliography was collected a very valuable contribution to the pharmacology of novocaine by Hatcher and Eggleston has appeared. In their paper they report two additional fatal cases of novocaine poisoning; the dosage in both cases probably did not exceed 20 mg. The paper is found in J. Pharmacol. Exper. Therap. 1916, v. 8, p. 385.

CONSIDERATION OF LABORATORY FINDINGS AND THEIR RELATION TO CLINICAL REPORTS.

The results of the laboratory experiments with cocaine and novocaine, when compared with the results obtained in the clinical use of these substances, show that man is relatively more susceptible to cocaine and novocaine than are laboratory animals. From animal experiments it is seen that the toxicity of these substances depends partly upon the manner and method of administration. The statement also seems to hold true for man. The untoward results that have been reported in the literature from the use of novocaine in operations about the head and face might well be accounted for by the fact that absorption may be very rapid, as for example in dental operations when the injection is made in a region well supplied with blood vessels, so that administration directly into the circulation is not unlikely to occur, and when injected in this way the toxicity is greater than when given subcutaneously. Individual susceptibility is marked in both animals and man. This may account for some of the fatalities reported in the literature.

FACTORS WHICH SHOULD BE CONSIDERED IN USE OF NOVOCAINE.

In addition to idiosyncrasy, age seems to be a factor in man in the production of fatal results with novocaine. In the three cases reported by Scandola, 1915, the ages of the men were 69, 75, and 80 years, respectively. In cases having low blood pressure, or cardiac disease, novocaine should be used with caution, inasmuch as in the laboratory experiments it has been shown to have a depressing effect upon the heart muscle when large doses are given.

The administration of hyoscine, previous to the use of a local anesthetic agent, is sometimes advised. If it is given before either cocaine or novocaine, it may act as a synergistic agent in depressing the respiration. In order to prevent the absorption of novocaine from the subcutaneous tissues, epinephrine is employed. Epinephrine is a relatively unstable agent, especially in alkaline solutions. It is not unlikely, therefore, that unless the epinephrine which is used with novocaine is active, general symptoms may arise from the administration of novocaine as a local anesthetic agent.

SUMMARY.

The melting point of novocaine, as determined from the examination of 10 samples used in this investigation, varied from 153° to 157° C. The relative toxicity of cocaine and novocaine, as shown by animal experiments, varies; the variation being dependent mainly upon the animal employed as test animal. The relative toxicity of cocaine and novocaine for various animals when given subcutaneously is as follows: For frogs (*Rana pipiens*) the ratio is 1.0 to 1.4; mice, 5.5 to 1; rats, 10 to 1; guinea pigs, 10 to 1; and rabbits 5.3 to 1. When given intravenously to rabbits, the ratio of toxicity of cocaine to novocaine is 3.9 to 1. When given intravenously the rate of administration is a factor in modifying the toxicity. The subcutaneous administration of large sublethal doses of novocaine in the dog and cat causes marked general symptoms which rapidly subside. The ratio of the toxicity of cocaine and novocaine for mice, when fed on cakes containing these substances, is much wider than when given in any other way, cocaine being about 50 times as toxic as novocaine. Feeding mice on sublethal doses of novocaine for a period of weeks did not seem to confer immunity to cocaine when the mice were fed on cocaine in the same way.

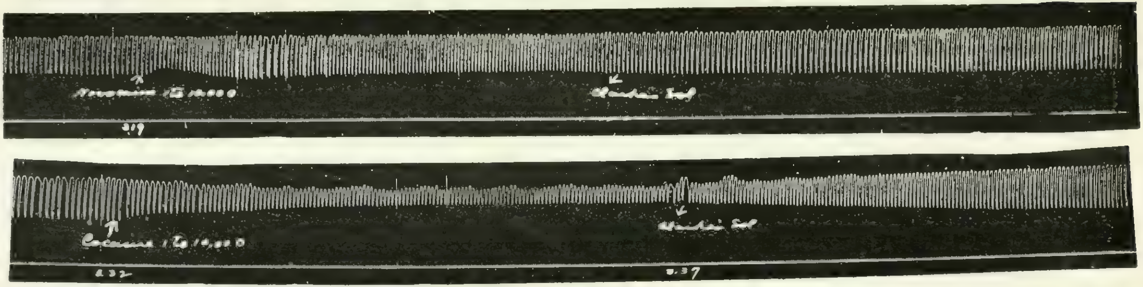
The effects of novocaine on the isolated heart of the frog resemble the effects produced by cocaine, both substances causing a decrease in rate of the heart and a decrease in the extent of systole. The relative toxicity on the heart of the frog as determined by perfusion experiments is less for novocaine than for cocaine. On smooth muscle, the effect of novocaine differs slightly from that produced by cocaine. On the isolated ureter of the dog, the isolated urinary bladder and stomach of the cat, and the isolated uterus of the rabbit, the effect of novocaine differs from that of cocaine only in being stimulating to a less degree when similar dilutions are used. On the isolated intestine of the rabbit, cocaine stimulates in dilute solutions, and in concentrated solutions depresses intestinal motility, whereas novocaine depresses it in any effective concentration. On the blood pressure and respiration, both cocaine and novocaine increase blood pressure and respiration in small doses and depress in large doses. When given subdurally, the relative toxicity of cocaine and novocaine is practically the same, as shown by the comparative effects on the blood pressure and respiration. Death in rabbits after cocaine or novocaine poisoning is usually respiratory, but with novocaine under certain conditions, death may be cardiac.

CONCLUSIONS.

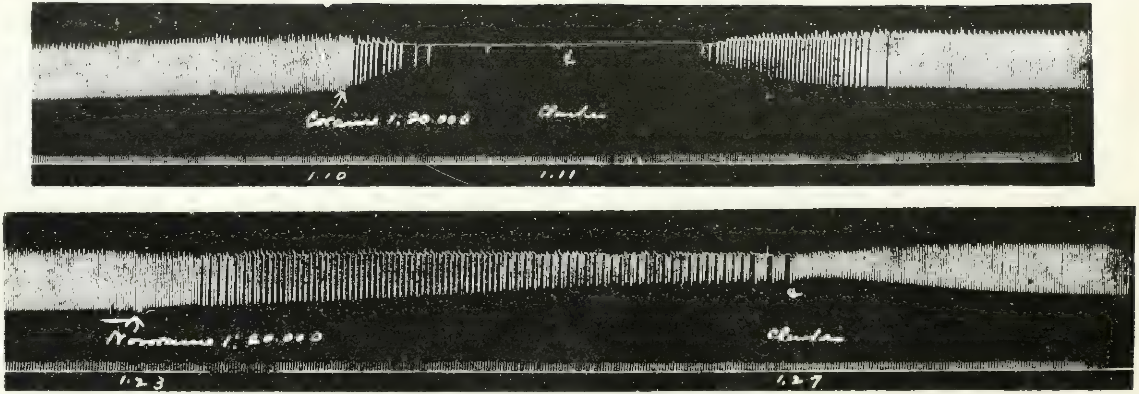
1. Novocaine is several times less toxic for laboratory animals than cocaine, the relative toxicity being dependent upon the method of administration as well as upon the animal used in making the determination.

2. Novocaine possesses many of the properties of cocaine as shown by experiments on the isolated heart, on smooth muscle, and by its effects on the circulation and respiration of anæsthetized animals.

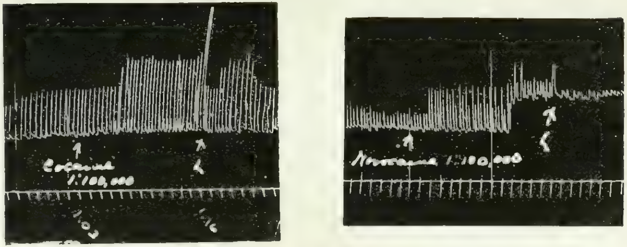
3. The depressing effect of novocaine on the blood pressure and respiration of animals makes it necessary to use caution in its ad-



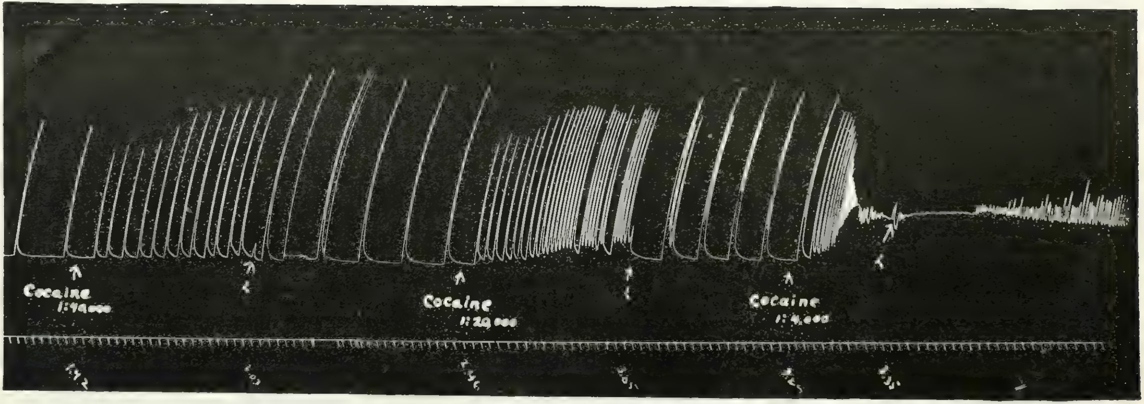
1. Tracings showing the comparative effects of 1 to 10,000 dilutions of cocaine and novocaine on the heart of *Rana esculenta* when perfused by Straub's method. Downstroke, systole; upstroke, diastole.



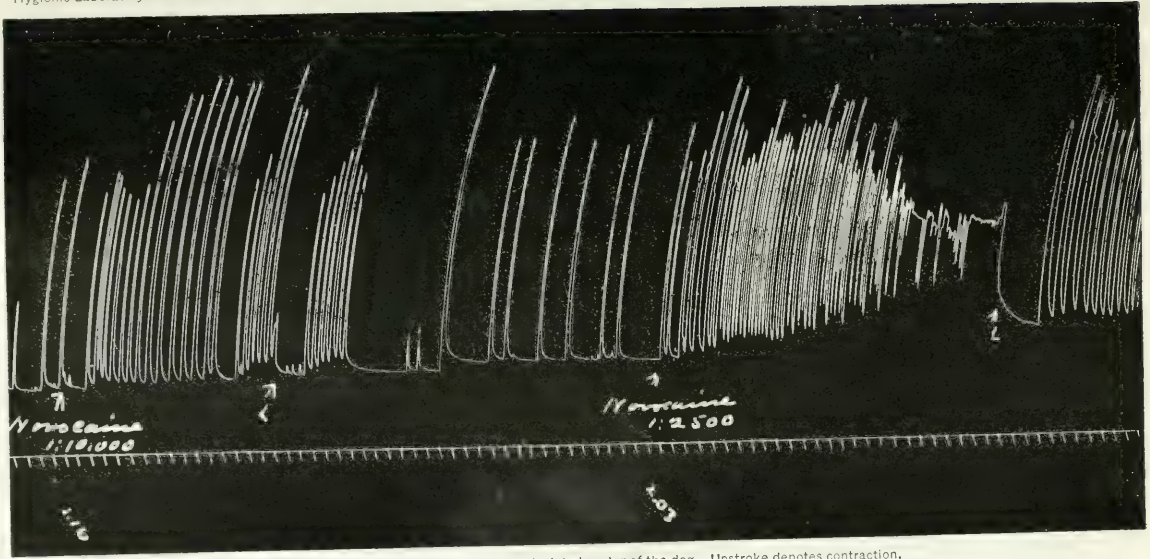
2. Tracings showing the comparative effects of 1 to 20,000 dilutions of cocaine and novocaine in Clarke's solution on the heart of *Rana pipiens* when perfused through the vena cava. Downstroke, systole; upstroke, diastole.



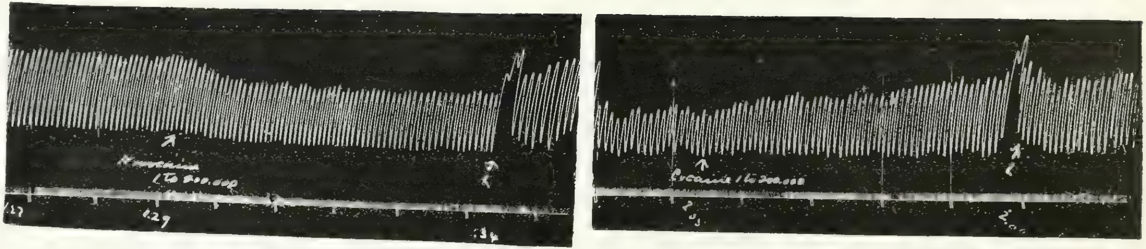
3. Tracings showing comparative effects of 1 to 100,000 dilutions of cocaine and novocaine on isolated ureter of the dog. Upstroke denotes contraction.



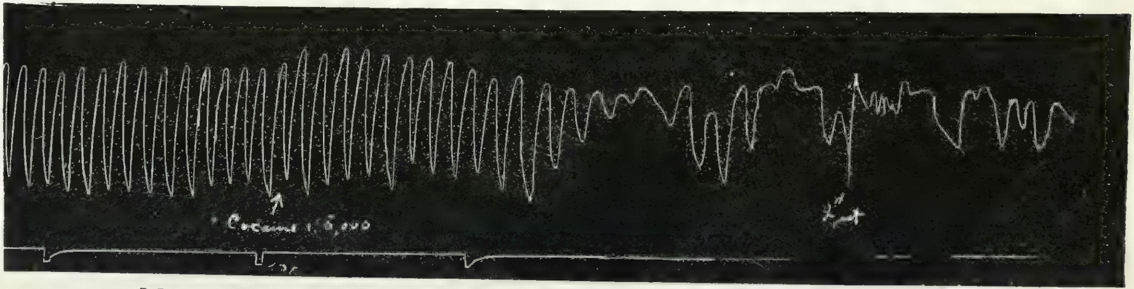
4. Tracing showing effects of various concentrations of cocaine on isolated ureter of the dog. Upstroke denotes contraction.



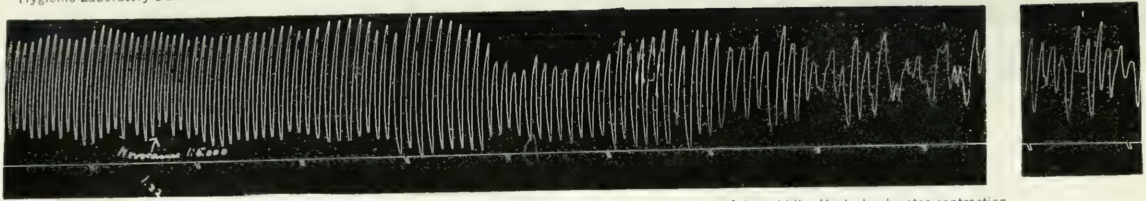
5. Tracing showing effects of novocaine on isolated ureter of the dog. Upstroke denotes contraction.



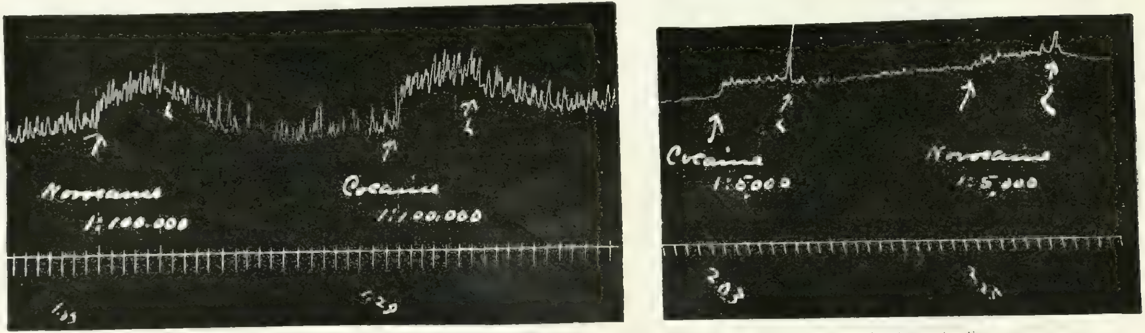
6. Tracings showing comparative effects of 1 to 200,000 dilutions of novocaine and cocaine on the isolated intestine of the rabbit. Upstroke denotes contraction. Note that cocaine causes an increase in the contraction while novocaine causes a decrease in the extent of the spontaneous contractions.



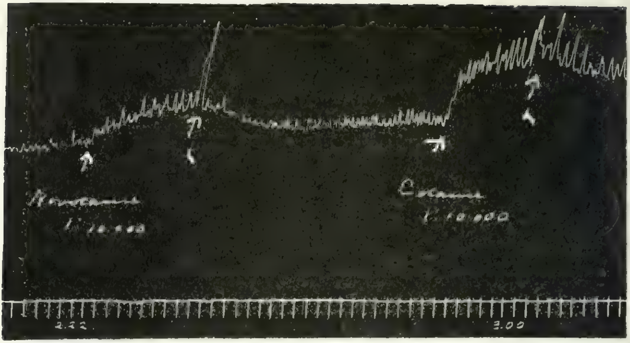
7. Tracing showing the effect of a 1 to 5,000 dilution of cocaine on the isolated intestine of the rabbit. Upstroke denotes contraction.



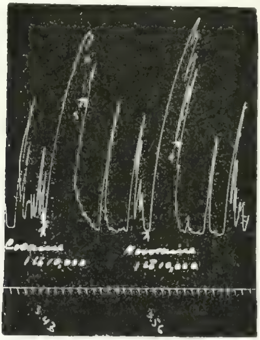
8. Tracing showing the effect of a 1 to 5,000 dilution of novocaine on the isolated intestine of the rabbit. Upstroke denotes contraction.



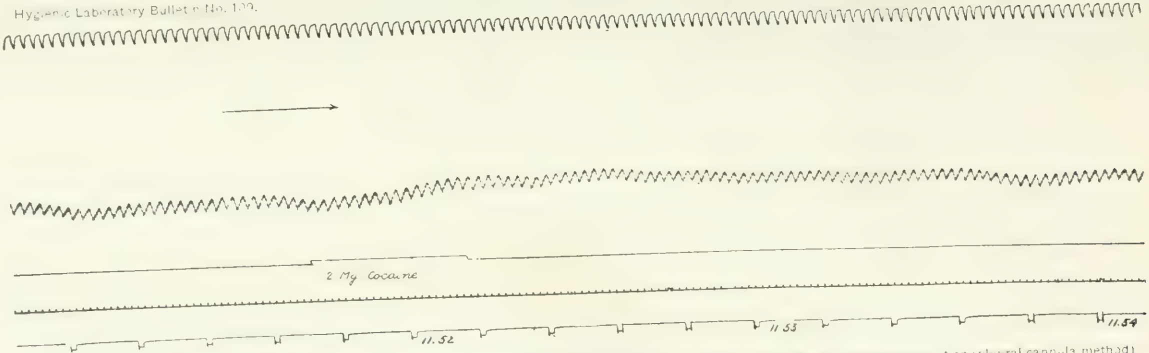
9. Tracings showing the effects of cocaine and novocaine on the isolated urinary bladder of the cat. Upstroke denotes contraction.



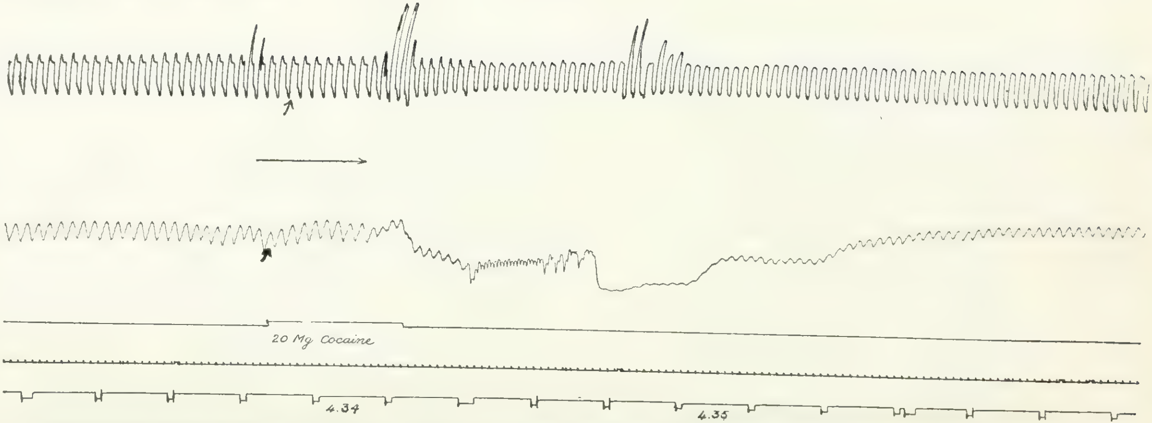
10. Tracing showing the effects of novocaine and cocaine on the isolated stomach (pyloric end) of the cat. Upstroke denotes contraction.



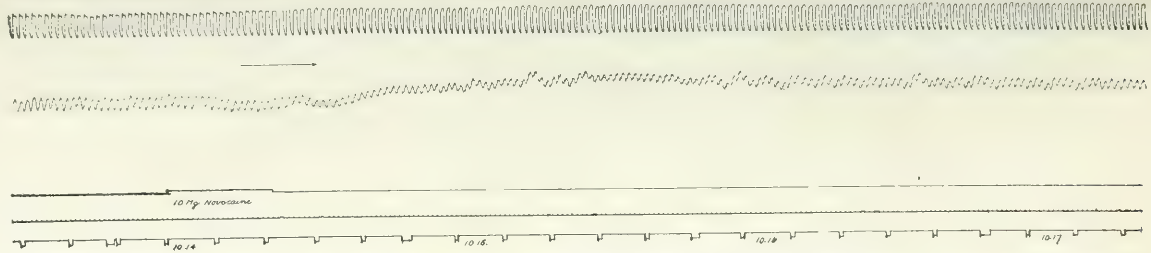
11. Tracing showing the effects of novocaine and cocaine on the isolated uterus of rabbit (non-pregnant). Upstroke denotes contraction.



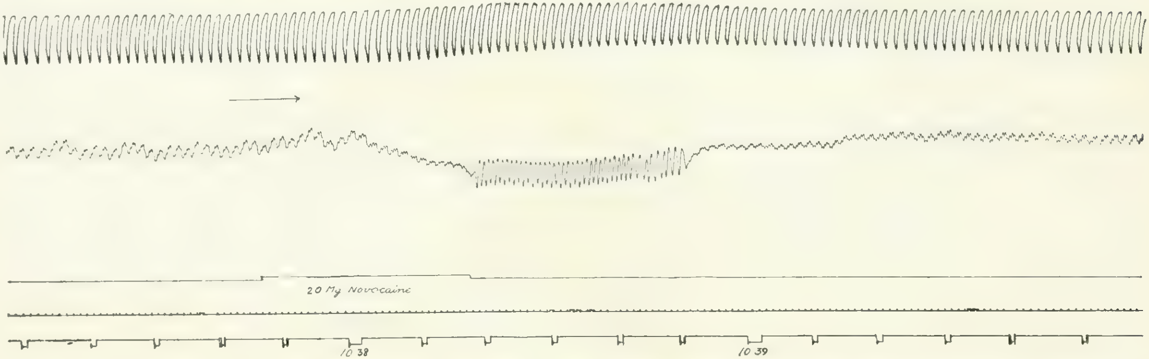
12. Tracings showing the effect of 2 mg. of cocaine injected intravenously into rabbit weighing 2.6 kg. Upper tracing shows effect on respiration (pleural cannula method) and lower shows effect on blood pressure. Note slight increase in rate of respiration and increase in height of blood pressure.



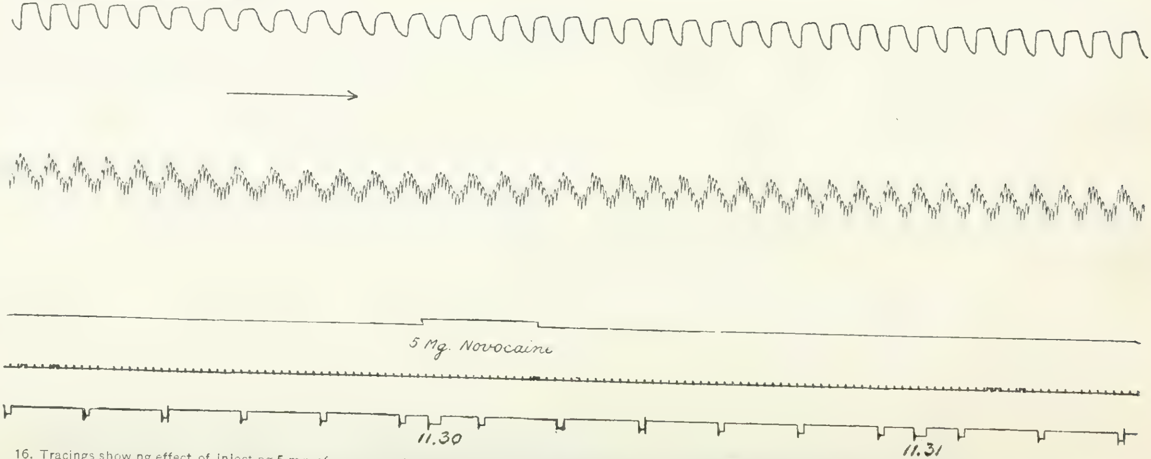
13. Tracings showing the effect of 20 mg. of cocaine injected intravenously in rabbit weighing 2.6 kg. Upper tracing shows effect on respiration as recorded by pleural cannula. Lower tracing shows effect on blood pressure.



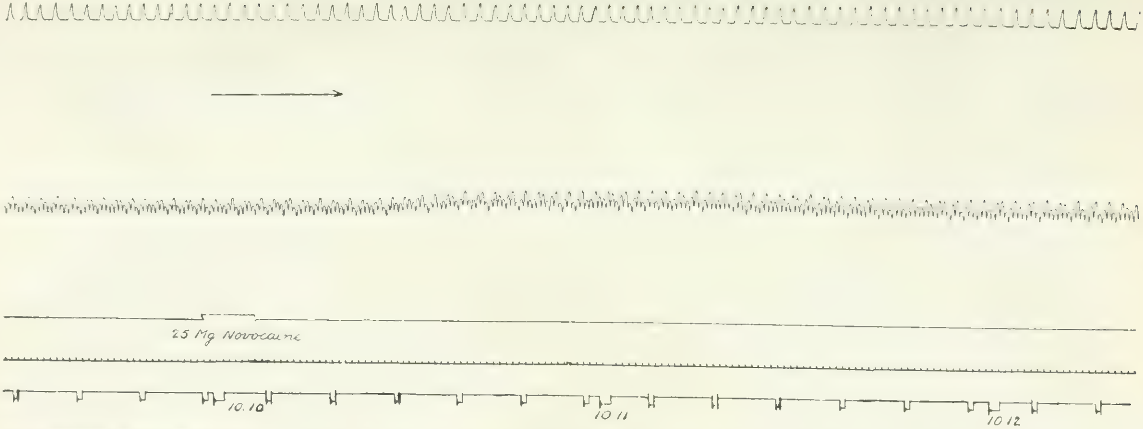
14. Tracings showing effect of injection of 10 mg. of novocaine intravenously in a rabbit weighing 2 kg. (First injection.) Upper tracing shows effect on respiration (pleural cannula method). Note increase in depth of respiratory movements. Lower tracing shows effect on blood pressure.



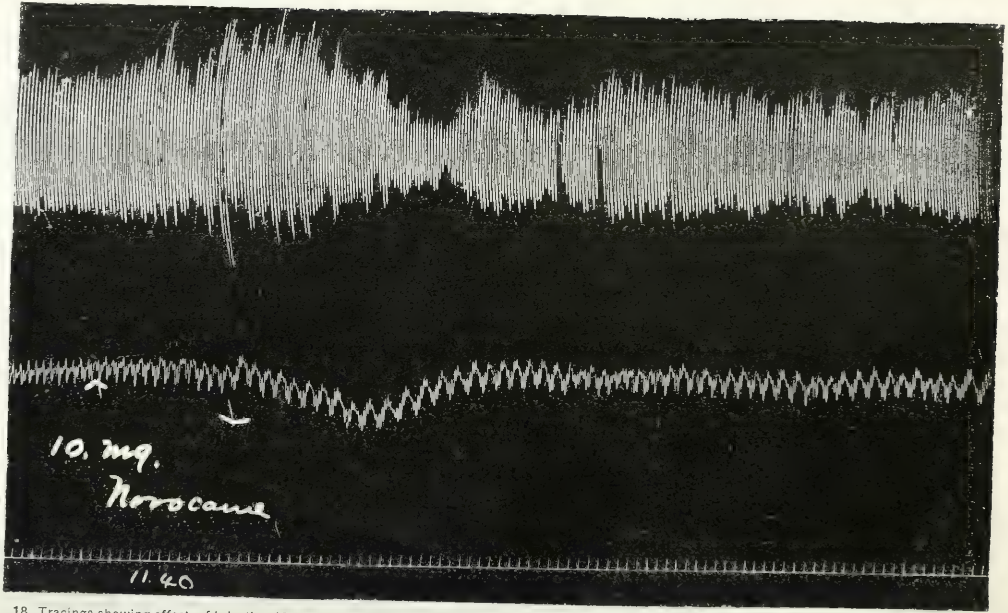
15. Tracings showing effect of injecting 20 mg. of novocaine in a 2.3 kg. rabbit. Upper tracing shows effect on respiration (pleural cannula method); lower tracing effect on blood pressure.



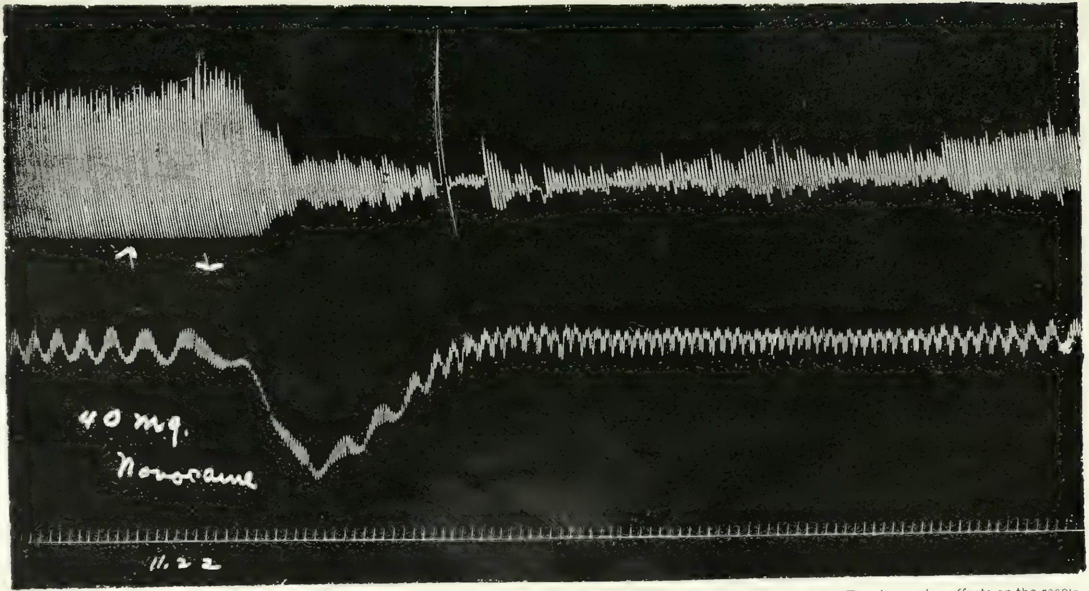
16. Tracings showing effect of injecting 5 mg. of novocaine into femoral vein of dog weighing 16 kg. Upper record shows effect on respiration as recorded by a pleural cannula and tambour; namely, a slight increase in the rate; also in the extent of chest movements. Lower record shows no effect on blood pressure.



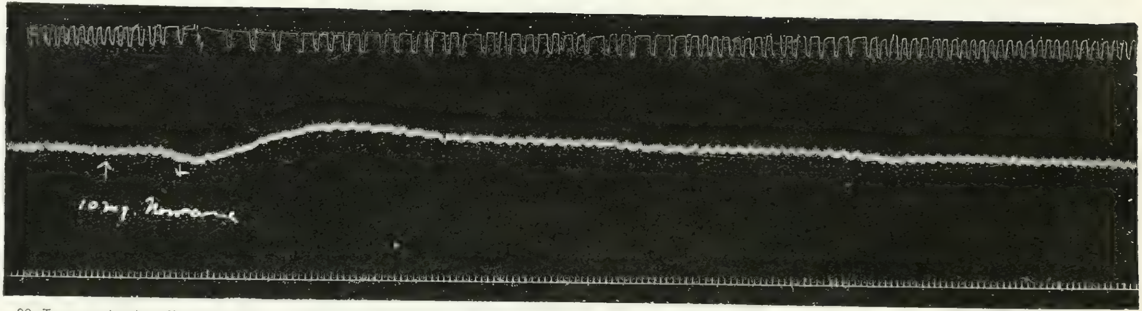
17. Tracing showing the effect of the intravenous injection of 25 mg. of novocaine (first injection) in a 5.45 kg. dog. Respiratory record (upper), obtained with pleural cannula, shows no effect on respiration while blood pressure record (lower) shows slight increase in pressure.



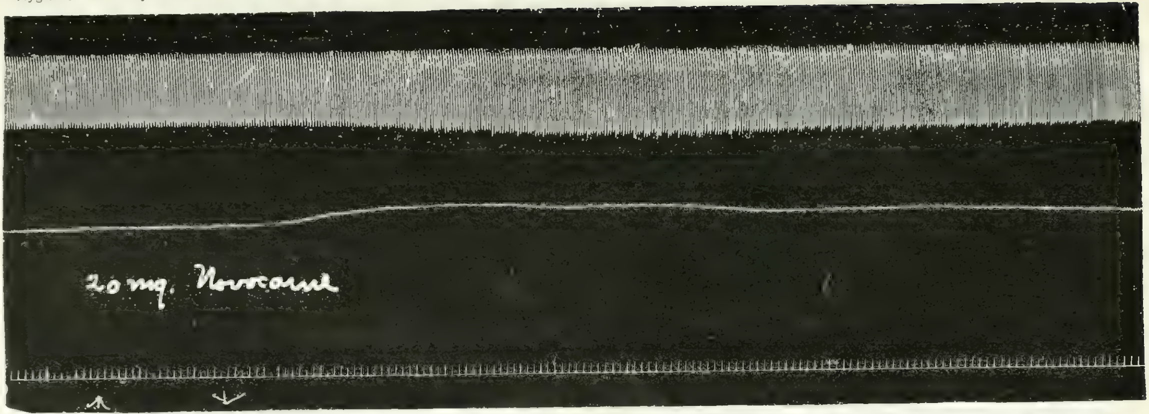
18. Tracings showing effect of injecting 10 mg. of novocaine into the femoral vein of dog weighing 9.5 kg. Upper record shows effect on respiration as recorded by tambour placed over diaphragm. Lower record shows effect on blood pressure. A slight increase in depth of respiration is noted followed by a decrease in depth, the blood pressure being lowered somewhat.



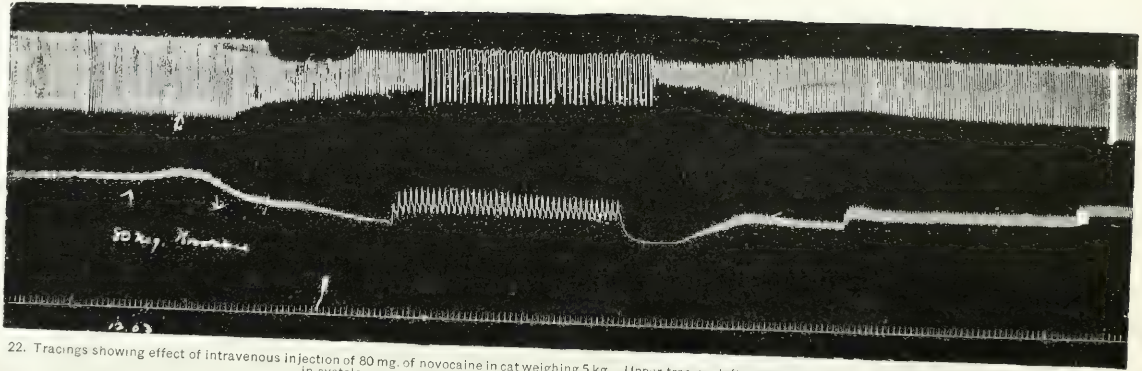
19. Tracings showing the effect of injecting 40 mg. of novocaine in the same dog which was used to obtain tracing in figure 18. The depressing effects on the respiration and blood pressure are more marked than in figure 18.



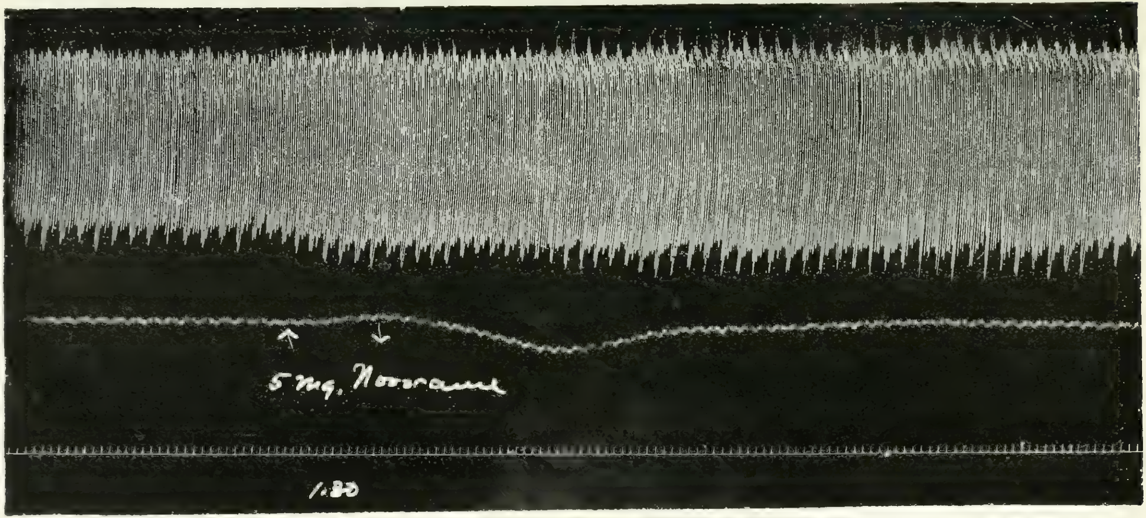
20. Tracings showing effect of intravenous injection of 10 mg. of novocaine in cat weighing 3 kg. Upper tracing shows effect on respiration (tambour method). Note decrease in rate of respiration. Lower tracing shows effect on blood pressure.



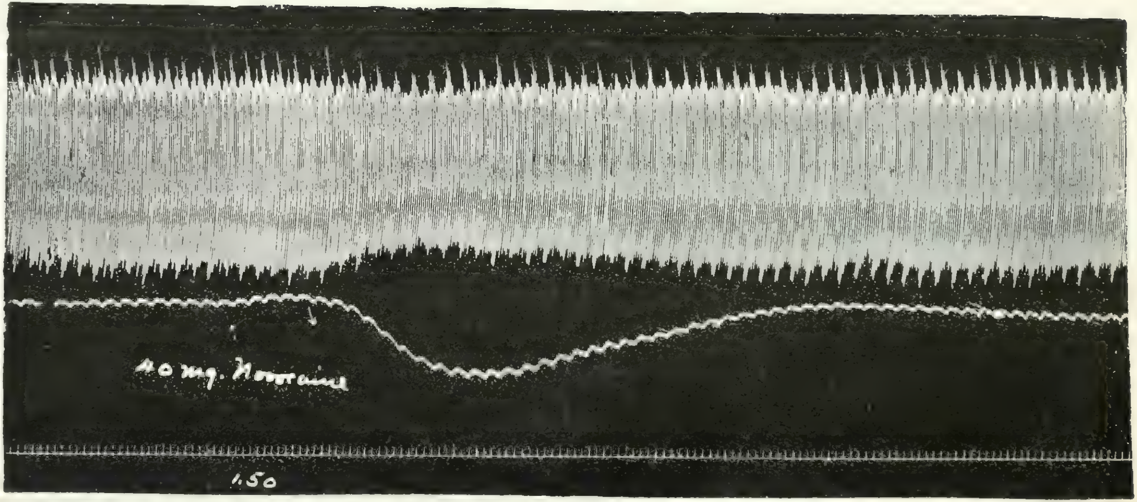
21. Tracings showing effect of intravenous injection of 20 mg. of novocaine on left ventricle and blood pressure of same cat used for figure 9. Upper tracing shows effect on left ventricle; lower tracing shows effect on blood pressure. Note increase in extent of systolic contraction.



22. Tracings showing effect of intravenous injection of 80 mg. of novocaine in cat weighing 5 kg. Upper tracing (left ventricle); lower tracing (blood pressure). Note decrease in systole and marked cardiac slowing, together with decrease in blood pressure.



23. Tracings showing effect of intravenous injection of 5 mg. of novocaine in dog weighing 9.5 kg. Upper tracing shows effect on left ventricle. Note increase in systole. Lower tracing shows effect on blood pressure. A fall in blood pressure occurs even though the heart contraction is stronger.



24. Tracings showing effect of intravenous injection of 40 mg. of novocaine in same dog as used for figure 23. Upper tracing (left ventricle). Lower tracing (blood pressure).

ministration in clinical cases in which the blood pressure is low or in which the heart is at fault.

4. Great care should be exercised in the injection of novocaine subcutaneously, in order to avoid its entrance into the circulation, thereby increasing its toxicity.

5. Individual susceptibility should always be considered in the administration of either cocaine or novocaine.

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HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

†No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis, Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

†No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

*No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

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*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

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Fitzgerald.

TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY—BULLETIN No. 110

SEPTEMBER, 1917

I. THE STANDARDIZATION OF ANTITYPHOID VACCINE.

By GEORGE W. McCOY.

II. A COLORIMETRIC METHOD FOR THE ESTIMATION OF THE CRESOL OR PHENOL PRESERVATIVE IN SERUMS.

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III. TOXICITY OF CERTAIN PRESERVATIVES USED IN SERUMS, VIRUSES, AND VACCINES.

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THE STANDARDIZATION OF ANTITYPHOID VACCINE.[1]

By George W. McCoy, *Director, Hygienic Laboratory.*

INTRODUCTION.

The use of killed cultures of *B. typhosus* as a prophylactic has become quite extensive, and the vaccine is generally recognized as an effective agent in preventing typhoid fever. It is not proposed to consider at present any phase of the subject of antityphoid vaccination other than the standardization of the prophylactic, nor will modified or mixed vaccines be dealt with in this paper.

Antityphoid vaccine is made by 14 commercial manufacturers holding Federal licenses, 11 State boards of health, and 3 municipal health departments.

So far as the writer is aware, there has been no official potency standard established for this product in this country or abroad. Publications by officers of the Army indicate that the prophylactic used by the Military Establishment will, when used to immunize rabbits, produce a serum that will give a high agglutinating titer, the figures given being 1 to 18,000–1 to 20,000.[2]

At the time our work was undertaken it was recognized that most of the problems that confronted us had been studied in connection with the several problems of typhoid immunization; but they appear not to have received attention from the point of view we had in mind, viz, the establishment of an official standard.

Throughout the work it was kept in mind that any method which was intended to be used by manufacturers should be as simple as possible and one which was subject to the fewest errors of interpretation. For this reason bactericidal, bacteriolytic, complement deviation, and opsonic index determinations were rejected and the agglutination reaction was adopted. Furthermore, there appeared no grounds for believing that any of the more complicated methods would yield more valuable information for the purpose in view than was to be derived from the one selected.

---

[1] Manuscript submitted for publication Jan. 6, 1917.

[2] Nichols. Trans. Int. Cong. Hyg., vol. 5, pt. 2, p. 797, 1912.

It was recognized at the outset that the fact that a given vaccine gave high immunity response in animals as measured by serological tests carried no guaranty that the same vaccine would be effective in immunizing man, and it was equally well understood that the failure of a vaccine to produce an immunity response in rabbits was not proof of its uselessness as an agent for human immunization. On the other hand, there is the ample and convincing testimony that a particular vaccine prepared in a certain manner does protect against typhoid infection and that this vaccine produces a definite immunity response in rabbits.

While we are not prepared to assert that a vaccine which complies with the test we have thought desirable to adopt is efficient, the fact that it is made in accordance with the procedure employed in making a known immunizing vaccine and from the same strain gives us reasonably secure grounds for believing that it is effective. On the contrary, if a vaccine fails to comply with the test there is good reason for suspecting that it has not been prepared properly. In other words, it seemed clear that a very considerable measure of security would accrue to those who used the agent if a definite potency standard were complied with by manufacturers.

The following facts in regard to the technic of the experiments reported in this paper hold good for all tests except where the contrary is specified in the text or in the tables:

The interval between injections was seven days. The injections were given into the subcutaneous tissue of the abdomen. The bleeding was made seven days after the last injection. The serum was separated and the agglutinations were set up on the day after the rabbits were bled. The tests were made with a physiological saline solution suspension of a one-day agar slant growth of the Rawling strain of *B. typhosus.* The reactions were done by the macroscopic method, using small test tubes (approximately 0.9 cm. in diameter), 0.5 c. c. of the serum dilution (double the strength of the dilution shown by the figures at the heads of the tables) being measured into the tube and an equal volume of the live typhoid suspension added. After shaking, the tubes were placed in an incubator at 37° C. for one hour and then in a cold room at 15° C. until the following morning, when the results were recorded. Absolute clearness of the supernatant fluid was indicated by + + + + and varying lesser degrees of agglutination and precipitation by + + +, + +, or +; 0 signifies complete lack of agglutination. A salt-solution control was always set up.

INVESTIGATION OF VACCINES IN USE IN THE UNITED STATES.

Investigation of commercial vaccines.—The following table will show the results of immunizing rabbits with several commercial vaccines. The bleedings were made 10 days after the last inoculation. The vaccines were all fresh. The serum dilutions were tested against emulsions made from two strains of *B. typhosus*.

A specimen of vaccine prepared at the Army Medical School and two prepared at the Hygienic Laboratory served as controls. Two rabbits were started on each vaccine, but in several instances one of them died before the immunization was completed.

TABLE I.

Product of manufacturer—	Rabbit—	Hopkins culture.			
		Serum dilution—			
		1 to 100	1 to 500	1 to 1,000	1 to 5,000
A	1	++++	++	0	0
	2	++++	+	0	0
B	1	+	0	0	0
	2	++++	++	0	0
C	1	++	0	0	0
	2	0	0	0	0
D	1	++++	+++	+	0
	2	++++	++	+	0
E	1	++++	++++	+++	0
	2	++++	++++	++++	++
F	1	++++	+++	+	0
	2	++++	++++	+++	++
G	1	+++	+	0	0
	2	++++	+++	+	0
H	1 [1] 2	++++	++++	+++	0
I	1 [1] 2	++++	++++	++	0
K	1	++++	++++	++	0
	2	++++	++	0	0
L	1	++++	+++	+	+
	2	++++	+++	+++	0
M	1	++++	++	+	0
	2	+++	++	+	0
N	1	++++	+++	+	0
	2	++++	++	+	0
O	1 [1] 2	+++	0	0	0
Army No. 169	1	++++	+++	+	0
	2	++++	++++	+++	0
Hyg. Lab. No. 7	1	++++	+++	++	+
	2	++++	++++	++	0
Hyg. Lab. No. 10	1	++++	++	+	0
	2	++++	++++	++	+

[1] Rabbit died.

TABLE I—Continued.

Product of manufacturer—	Rabbit	Rawling culture.			
		Serum dilution—			
		1 to 100	1 to 500	1 to 1,000	1 to 5,000
A	1	++++	+++	+	0
	2	++++	+	+	0
B	1	+	0	0	0
	2	+++	+	0	0
C	1	++	+	+	0
	2	++	0	0	0
D	1	++++	++++	+++	0
	2	++++	+++	+	0
E	1	++++	++++	+++	0
	2	++++	++++	++++	++
F	1	++++	+++	+++	0
	2	++++	++++	++++	+++
G	1	++++	++	0	0
	2	++++	+++	++	0
H	1	++++	++++	++++	+
	[1]2				
I	1	++++	++++	+++	0
	[1]2				
K	1	++++	+++	+++	0
	2	++++	+++	+++	0
L	1	++++	++++	+++	+
	2	++++	++++	+++	0
M	1	++++	++	+	0
	2	++++	+++	+	0
N	1	++++	+++	++	0
	2	++++	++	+	0
O	1	++	+	0	0
	[1]2				
Army No. 169	1	++++	++	+	0
	2	++++	++++	+++	+
Hyg. Lab. No. 7	1	++++	+++	+	0
	2	++++	++++	++	0
Hyg. Lab. No. 10	1	++++	+++	+	0
	2	++++	++	0	0

[1] Rabbit died.

It will be observed that three of these vaccines (B, C, and O) fall notably below the average and that several of the others fall distinctly below the controls, while two (E and F) made a better showing than the controls, and several were about equal to the controls.

This table also shows that the Rawling culture, which was the homologous strain for the controls and for most of the commercial products, gave readings very similar to those given by the Hopkins strain.

A second test was made of the three vaccines B, C, and O, which had made the poorest showing in the preceding test. At this time two specimens of vaccine of manufacturer B were available. As controls, two vaccines made at the Hygienic Laboratory were employed. The rabbits were inoculated and bled as in the previous experiment. The results for a single antigen (Rawling) are shown:

TABLE II.

Product of manufacturer—	Rabbit—	Rawling culture.			
		Serum dilution –			
		1 to 100	1 to 500	1 to 1,000	1 to 5,000
B(1)	1	++++	+	0	0
	2	++++	+++	++	0
B(2)	1		0	0	0
	2	+++	+	0	0
C	1	++	0	0	0
	2	+	0	0	0
O	1	+	0	0	0
	2	+	0	0	0
Hyg. Lab. No. 10	1	++++	++	0	0
	2	++++	+++	0	0
Hyg. Lab. No. 11	1	++++	++++	++	0
	[1] 2				

[1] Died.

These results clearly show that the products C and O do not come up to the controls and that specimen 2 of manufacturer B is also deficient in agglutinin production.

Vaccines made by State laboratories.—Tests were then made with vaccines produced by various State laboratories. In the first experiment the products of seven States were available. These gave the following results, Army and Hygienic Laboratory products being used as controls:

TABLE III.

Product of State—	Rabbit—	Hopkins culture.				
		Serum dilution—				
		1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
A	1	++++	++++	+++	+++	+++
	2	+++	+++	+++	++	+
	3	+++	+++	+++	+++	++
B	1	0	0	0	0	0
	2	+++	++	0	0	0
	3	++	++	+	0	0
C	1	+++	+++	+++	++	++
	2	++++	+++	+++	+++	++
	3	++++	++++	+++	+++	++
D	1	+++	+++	+++	++	+
	2	++++	+++	++++	+++	++
	3	++++	+++	+++	++	+
E	1	+++	+++	++	+	+
	2	++	+++	+++	++	+
	3	+++	+++	Lost.	++	+
F	1	++++	+++	+++	++	++
	2	+++	+++	+++	++	++
	3	+++	+++	++	++	+
G	1	++++	++++	+++	++	+
	2	++++	++++	+++	++	+
	3	++++	+++	+++	+	0
Army No. 172	1	+++	++++	++++	+++	++
	2	+++	++++	+++	+++	+
	3	+++	+++	+++	+++	+++
Hyg. Lab. 10	1	+++	+++	++++	+++	++
	2	+++	+++	+++	+++	+
Hyg. Lab. 13	1	+++	+++	+++	+++	++
	2	++++	+++	+++	+++	++

Table III—Continued.

Product of State	Rabbit	Rawling culture.				
		Serum dilution—				
		1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
A	1	++++	++++	++++	+++	+++
	2	++++	++++	+++	++	+
	3	++++	++++	++++	+++	++
B	1	0	0	0	0	0
	2	++	+	0	0	0
	3	++	+	+	0	0
C	1	++++	++++	++++	+++	++
	2	++++	++++	++++	+++	++
	3	++++	++++	+++	++	++
D	1	++++	+++	+++	+	+
	2	++++	++++	++++	+++	+++
	3	++++	+++	+++	++	++
E	1	++++	+++	++	+	+
	2	++++	++++	++++	++	+
	3	++++	++++	+++	++	+
F	1	++++	++++	++++	++++	+++
	2	++++	++++	++++	++++	+++
	3	+++	+++	++	+	+
G	1	++++	+++	++	+	0
	2	++++	+++	+	+	0
	3	++++	+++	0	0	0
Army No. 172	1	++++	++++	++++	++++	+++
	2	++++	+++	+	+	0
	3	++++	++++	++++	+++	+++
Hyg. Lab. 10	1	++++	++++	++++	+++	++
	2	++++	+++	++	+	0
Hyg. Lab. 13	1	++++	++++	+++	+++	++
	2	++++	++++	++++	++	++

It will be observed that the product of State B is notably deficient. Specimens from four other States which were received at a later date gave the following results, using a single strain as antigen and using an Army vaccine as a control:

Table IV.

Product of State—	Rabbit—	Rawling culture.					
		Serum dilution—					
		1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
H	1	++++	++++	++++	++	0	0
	2	++++	++++	+	0	0	0
	3	++++	++++	++++	+++	++	0
I	1	++++	++++	++++	+++	+++	++
	2	++++	++++	++++	+++	+	0
K	1	++++	++++	++++	++++	+++	+
	2	++++	++++	+++	+	0	0
L	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
Army No. 172	1	++++	++++	++++	++++	+++	+
	2	++++	++++	++++	++++	+++	++

Vaccine L showed no agglutinin production whatever. Indeed there were some grounds for believing that little or no *B. typhosus* entered into it—an example of what may occur when the manufacture of this prophylactic is not properly supervised and is not controlled by adequate tests.

Vaccines made by municipal laboratories.—Vaccines prepared by three municipal health department laboratories were tested with the following results, a Hygienic Laboratory preparation being used as a control. All these fell somewhat below the control, but were not seriously deficient.

TABLE V.

Product of city—	Rabbit—	Rawling culture.					
		Dilutions of serum—					
		1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
A	1	++++	+++	++	+	0	0
	2	++++	+++	+++	++	+	+
	3	++++	+++	+++	++	+	+
B	1	Died.					
	2	++++	+++	++	+	+	0
	3	++++	+++	++	+	+	0
C	1	++++	+++	++	+	+	0
	2	++++	+++	++	+	0	0
	3	++++	+++	+	0	0	....
Control 18A	1	++++	+++	++	++	++	+
	2	++++	++++	+++	++	++	+
	3	++++	+++	+++	++	++	++

INVESTIGATION OF CERTAIN FACTORS CONCERNED.

In order to determine the influence of several factors on the production of agglutinin, an experiment was designed to determine the relative agglutinin production when the three doses of the vaccine were given at various intervals and when all were given at one dose; when given intravenously and when given subcutaneously, and when the animals were bled at various times after the completion of the series of injections. The final (or only) dose of vaccine was given to all animals on the same day. A vaccine of our own making was used for the tests and a Rawling culture for the agglutinations. The chart following shows the result of the experiment.

TABLE VI.

Intervals between inoculations.	Mode of inoculation.	Rabbit	Agglutination titer of serum 48 hours after last (or only) inoculation.					
			1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
7 days	Subcutaneous	1	+++	+++	+++	++	0	0
		2	++++	++++	++++	+++	++	0
		3	++++	+++	+++	+++	++	0
	Intravenous	1	Died.					
		2	++++	++++	++++	++++	++++	+++
		3	Died.					
5 days	Subcutaneous	1	++++	+++	+++	++	0	0
		2	Died.					
		3	++++	++++	++++	++	+	0
	Intravenous	1	Died.					
		2	Died.					
		3	++++	++++	++++	+++	+++	+++
3 days	Subcutaneous	1	++++	++++	++++	++++	+++	0
		2	++++	++++	++++	+++	+	0
		3	++++	++++	++++	+++	++	0
	Intravenous	1	Died.					
		2	Died.					
		3	++++	++++	++++	++++	++++	+++
1 day	Subcutaneous	1	+	0	0	0	0	0
		2	+++	0	0	0	0	0
		3	+	0	0	0	0	0
	Intravenous	1	+++	++	+	0	0	0
		2	+++	++	+	0	0	0
		3	Died.					
3 doses at single injection.	Subcutaneous	1	0	0	0	0	0	0
		2	+	0	0	0	0	0
		3	0	0	0	0	0	0
	Intravenous	1	+	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0

Intervals between inoculation.	Mode of inoculation.	Rabbit—	Agglutination titer of serum 5 days after last (or only) inoculation.					
			1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
7 days	Subcutaneous	1	++++	++++	++++	+++	++	0
		2	++++	++++	++++	+++	++	+
		3	++++	++++	++++	++++	+++	++
	Intravenous	1						
		2	++++	++++	++++	++++	++++	++++
		3						
5 days	Subcutaneous	1	++++	++++	+++	+++	+++	+
		2						
		3	++++	++++	++++	+++	+	0
	Intravenous	1						
		2						
		3	++++	++++	++++	++++	++++	+++
3days	Subcutaneous	1	++++	++++	+++	+++	++	+
		2	++++	++++	++++	+++	++	0
		3	++++	++++	++++	+++	++	0
	Intravenous	1						
		2						
		3	++++	++++	++++	+++	++	+
1 day	Subcutaneous	1	++	+	0	0	0	0
		2	++++	++++	++	0	0	0
		3	++++	+++	+	0	0	0
	Intravenous	1	++++	++++	++++	+++	0	0
		2	++++	+++	++	0	0	0
		3						
3 doses at single injection.	Subcutaneous	1	++++	++++	+++	++	0	0
		2	+++	+	0	0	0	0
		3	++++	++++	++	0	0	0
	Intravenous	1	++++	++++	+++	+	0	0
		2	++++	++++	+++	++	0	0
		3	++++	++++	+++	++	0	0

TABLE VI—Continued.

Intervals between inoculations.	Mode of inoculation.	Rabbit—	Agglutination titer of serum 8 days after last (or only) inoculation.					
			1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,000
7 days	Subcutaneous	1	++++	++++	++++	+++	++	0
		2	++++	++++	++++	+++	+++	+
		3	++++	++++	++++	++++	++	++
	Intravenous	1						
		2	++++	++++	++++	++++	++++	+++
		3						
5 days	Subcutaneous	1	++++	++++	++++	++++	++	+
		2						
		3	++++	++++	+++	+++	+	0
	Intravenous	1						
		2						
		3	++++	++++	++++	++++	++++	++++
3 days	Subcutaneous	1	++++	++++	++++	++++	++++	++
		2	++++	++++	++++	+++	+	0
		3	++++	++++	++++	+++	++	+
	Intravenous	1						
		2						
		3	++++	++++	++++	++++	++	+
1 day	Subcutaneous	1	++++	+++	+	0	0	0
		2	++++	++++	+++	++	0	0
		3	+++	++	++	+	0	0
	Intravenous	1	++++	++++	++++	++++	++	+
		2	++++	++++	+++	++	+	0
		3						
3 doses at single injection.	Subcutaneous	1	++++	++++	++++	+++	+	0
		2	++++	++++	++	+	0	0
		3	++++	++++	+++	++	++	+
	Intravenous	1	++++	++++	++++	+++	0	0
		2	++++	++++	++++	+++	+	0
		3	++++	++++	++++	++++	+++	+

Intervals between inoculations.	Mode of inoculation.	Rabbit—	Agglutination titer of serum 12 days after last (or only) inoculation.					
			1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,000
7 days	Subcutaneous	1	++++	++++	++++	+++	++	+
		2	++++	++++	++++	+++	+++	++
		3	++++	++++	++++	+++	+++	++
	Intravenous	1						
		2	++++	++++	++++	++++	++++	++++
		3						
5 days	Subcutaneous	1	++++	++++	++++	+++	+++	++
		2						
		3	++++	++++	++++	+++	++	0
	Intravenous	1						
		2						
		3	++++	++++	++++	++++	++++	+++
3 days	Subcutaneous	1	++++	++++	++++	++++	++++	+++
		2	++++	++++	++++	+++	++	0
		3	++++	++++	++++	++++	++	++
	Intravenous	1						
		2						
		3	++++	++++	++++	++++	++++	+++
1 day	Subcutaneous	1	Died.					
		2	++++	++++	++	+	0	0
		3	++++	+++	++	+	0	0
	Intravenous	1	++++	++++	++++	+++	++	+
		2	++++	++++	+++	+++	++	+
		3						
3 doses at single injection.	Subcutaneous	1	++++	++++	+++	+++	++	0
		2	++++	+++	++	++	0	0
		3	++++	++++	+++	+++	++	+
	Intravenous	1	++++	++++	++++	+++	+++	++
		2	Died.					
		3	++++	++++	++++	+++	++	++

TABLE VI—Continued.

Intervals between inoculations.	Mode of inoculation.	Rabbit—	Agglutination titer of serum 16 days after last (or only) inoculation.					
			1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
7 days	Subcutaneous	1	++++	++++	++++	++++	++	+
		2	++++	++++	++++	++++	++	+
		3	++++	++++	++++	++++	+++	++
	Intravenous	1						
		2	++++	++++	++++	++++	++++	++++
		3						
5 days	Subcutaneous	1	++++	++++	++++	+++	++	+
		2						
		3	++++	++++	+++	++	0	0
	Intravenous	1						
		2						
		3	++++	++++	++++	++++	++++	+++
3 days	Subcutaneous	1	++++	++++	++++	++++	++++	+++
		2	++++	++++	+++	+++	+	0
		3	++++	++++	+++	+++	+++	+
	Intravenous	1						
		2						
		3	++++	++++	++++	++++	++++	++
1 day	Subcutaneous	1						
		2	+++	++	+	+	0	0
		3	+++	+	+	+	0	0
	Intravenous	1	++++	++++	++++	+++	++	++
		2	++++	+++	+++	++	+	+
		3						
3 doses at single injection.	Subcutaneous	1	++++	++++	++++	+++	+++	+
		2	++++	+++	+++	+	+	0
		3	++++	++++	+++	+++	+	0
	Intravenous	1	++++	++++	++++	+++	++	+
		2						
		3	++++	++++	++++	+++	++	+

Without laying too much emphasis on the results, it seems clear that we may conclude:

1. That intravenous inoculation leads to earlier and higher agglutinin development than does the subcutaneous method.

2. That an interval between inoculations of 3 days seems as effective as one of 5 or 7, and that a period of 5 days after the inoculation gives nearly complete agglutinin response when the interval between inoculations has been 3 days or more.

3. When the vaccine is given at a single dose or at intervals of 24 hours, a period of 8 days gives almost complete response.

Spacing of injections.—In order to determine whether spacing the injections at rather long intervals would lead to an agglutinin response different from that due to injections at shorter intervals, a series of rabbits was inoculated at intervals of 12 days and another series at intervals of 4 days, the last injection in each case being given on the same day. The vaccine used was a Hygienic Laboratory product. Seven days after the last inoculation the serums gave the following results:

TABLE VII.

Intervals of injections.	Rabbit—	Rawling strain.					
		Dilutions of serum—					
		1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
12 days	1	++++	++++	+++	++++	+++	+
	2	++++	++++	++++	+	+	+
	3	++++	++++	++++	+++	++	+
4 days	1	++++	++++	+++	+	+	0
	2	++++	++++	+++	++	++	+
	3	++++	++++	++++	+++	++	+

It does not appear that there is any striking difference between the results obtained by the two methods.

Variation in dosage of vaccine.—In order to determine what variation in agglutinin response might occur by varying the dose of vaccine used, three rabbits were given one-tenth of the usual human dose, three one-half the dose, and three the full dose. The three doses were given at intervals of five days and the blood drawn five days after the last injection. It was a matter of interest to observe that these differences in dosage made relatively little difference in agglutinin production. Therefore it was inferred that no great exactness in dosage was necessary. The vaccine used in this and all following experiments was one prepared at the Hygienic Laboratory. The results of the experiment are shown here.

TABLE VIII.

Dosage.	Rabbit—	Rawling culture.					
		Dilutions of serum—					
		1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
$\frac{1}{10}$ full dose	1	++++	++++	++++	+++	0	0
	2	++++	++++	+++	++	0	0
	3	++++	++++	++++	+++	0	0
$\frac{1}{2}$ full dose	1	++++	++++	+++	+++	0	0
	2	++++	++++	++++	++++	+++	++
	3	++++	++++	++++	+++	++	0
Full dose	1	++++	++++	+++	+	0	0
	2	++++	++++	++++	+++	++	0
	3	++++	++++	++++	++++	+++	+

Subcutaneous and intramuscular inoculation.—During the course of the investigation it occurred to us that material differences in immunization response might occur if the vaccine should be given by accident into the muscular tissues of the animal. To determine the facts, a series of six rabbits was inoculated with the three doses at intervals of five days, half being given the vaccine into the lumbar muscles, the remainder being given the material in the usual way, subcutaneously on the abdomen. Nine days after the last inoculation the following results were obtained:

Tabbe IX.

Method of injection.	Rawling culture.					
	Dilutions of serum—					
	1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
Subcutaneously....	++++	++++	++++	++++	+++	++
	++++	++++	+++	++	+	0
	++++	++++	+++	++	+	0
Intramuscularly...	++++	++++	++++	++++	++++	++++
	++++	++++	++++	++++	+++	++
	++++	++++	++++	++++	++	+

These results tend to indicate, if anything, that the intramuscular inoculations give the higher agglutinin response.

Effect of heat on the vaccine.—It is known that high temperatures are unfavorable to the vaccine and a single experiment, in which various degrees of heat were applied for one hour, shows that excessive heat unfavorably influences agglutinin production. The injections were given at intervals of five days and the serum tested 12 days after the last injection.

Table X.

Heated to—	Rabbit—	Dilutions of serum—					
		1 to 50	1 to 100	1 to 200	1 to 400	1 to 800	1 to 1,600
53° C..................	1......	++++	++++	++++	++	+	+
	2......	++++	++++	++++	+++	++	+
	3......	++++	++++	++++	+++	++	+
60° C.................	1......	++++	++++	++	++	+	0
	2......	++++	++++	++++	+++	+++	++
80° C..................	1......	++++	+++	++	+	+	0
	2......	+++	++	+	0	0	0
	3......	+++	++	+	0	0	0
100° C.................	1......	0	0	0	0	0	0
	2......	0	0	0	0	0	0
	3......	0	0	0	0	0	0

So far as we can judge, a temperature in excess of 60° C. is required materially to influence agglutinin production. In the manufacture of the product at present the degree of heat that is thought desirable is about 53° to 54° C. This is not sufficient to kill all of the microorganisms, the survivors being destroyed by the preservative which is later added.

The importance of this in the present connection is the fact that the use of a degree of heat necessary to effect sterilization and thus cover defects in the technic of manufacture might result in a low potency showing.

Variation of rabbits.—An inspection of the results of the experiments makes it plain that there is considerable individual variation among rabbits in the production of antityphoid agglutinin. It is believed that errors due to this may be overcome by the use of several animals for each test. Animals of about the same weight should be used for the test and the control.

Natural antityphoid agglutinin in rabbit blood.—A preliminary test made with serum from 20 rabbits, using all of the typhoid cultures available, showed that in a final dilution of 1 to 10 in the majority of cases more or less complete agglutination occurred. Later tests have shown that occasionally agglutination may occur in dilutions of normal rabbit serum as high as 1:100.

In order to determine whether the presence of natural agglutinin had any appreciable influence on the development of agglutinin when these rabbits were submitted to inoculation with the vaccine, three were chosen which showed little or no natural agglutinin and three which showed a considerable amount. These six rabbits were immunized with a vaccine (H. L. 15) and their serum tested in the usual manner. The results are shown in the accompanying table.

TABLE XI.

Serum used.	Rabbit—	Rawling culture.			
		Titration of normal rabbit serum.			
		Serum dilution—			
		1 to 5.	1 to 10.	1 to 20.	1 to 40.
Serum low in natural agglutinin	1	++	0	0	0
	2	+++	+	0	0
	3	0	0	0	0
Serum high in natural agglutinin	1	+++	++	0	0
	2	++++	++	0	0
	3	++++	+++	++	0

Serum used.	Rabbit—	Rawling culture.					
		Serum of same animals after aggultination.					
		Serum dilution—					
		1 to 50.	1 to 100.	1 to 200.	1 to 400.	1 to 800.	1 to 1,600.
Serum low in natural agglutinin	1	++++	++++	++++	+++	+++	+
	2	++++	++++	++++	++++	++	0
	3	++++	++++	+++	++	0	0
Serum high in natural agglutinin	1	++++	++++	++++	++++	++	0
	2	++++	++++	++++	+++	+++	0
	3	++++	++++	++++	+++	++	+

It will be observed that there is no striking difference in the results shown by the two groups of rabbits. Rabbit No. 3 in the first group, which showed no natural agglutinin for this antigen, was, however, the poorest agglutinin producer in the lot. Two other tests of the serum of the animals after immunization gave substantially the same results as shown above. It was felt that, for practical purposes, the presence of natural agglutinin could be ignored.

APPLICATION OF RESULTS.

Throughout our experiments it was recognized that, even with great exactness in technic, it would be difficult for persons working in different laboratories to obtain agglutination titers that would closely approximate each other. The exact concentration of the suspension used in making the test is particularly difficult to duplicate, and, above all, the personal equation in reading results would be certain to lead to varying results. Therefore, it seemed that the best solution of this problem lay in furnishing to manufacturers for control of the tests on their products a carefully prepared vaccine which was known to give satisfactory agglutinin production. The Hygienic Laboratory is prepared to furnish such a vaccine.

It is believed that the choice of the method of administering it, the time interval between doses, and the precise technic of carrying out the test may be left to the individual worker, the sole requirement in this respect being that the commercial product shall approximate in agglutinin production the standard furnished.

Intravenous inoculation gives a higher agglutinin response than does the subcutaneous method, but has so high a mortality that it is not recommended, the subcutaneous method being preferred.

As it is desired to make the time required for the test as short as possible, it is believed that there is no objection to giving the entire amount of material (2.5 c. c.) at a single dose, the controls being given the test vaccine in the same manner, bleedings to be made after 8 days.

Using a control, the temperature at which the reaction is carried out is a matter of comparatively little consequence.

At least three rabbits should be used for the test and three for the control. Some rabbits seem to be incapable of yielding a marked agglutinin response and possibly some are unduly sensitive, but by using several the exceptional one can be detected and rejected.

The strain of B. typhosus used almost exclusively by American manufacturers for making the vaccine is that known as the Rawling culture, and it is this which should be used for preparing the emulsion for the tests. This particular strain may be no more efficient for preparing a vaccine than others, but it has been so extensively used, and generally with such satisfactory results, that there seems to be no good reason for employing another.

The procedure used at this laboratory for making and testing typhoid vaccine is given below. It is believed that any material abridgment of or variation from the procedure will result in uncertainty as to the quality of the final product.

It is a modification of that given by Nichols[1] as employed at the United States Army Medical School.

APPENDIX.

DIRECTIONS FOR MAKING TYPHOID PROPHYLACTIC.

CULTURE.

1. Plate the Rawling culture on at least one set of Endo plates; incubate 24 hours.
2. Examine colonies and mark at least three which have typical appearance and are large and well separated.
3. From each colony make a transfer to three tubes. (1) Two broth tubes (2) a stab and slant of Russell's double sugar agar. Keep corresponding tubes together; incubate 24 hours.
4. Examine growth and make macroscopic agglutinations by adding one drop of antityphoid serum of high titre to one broth tube inoculated from each colony. Incubate one-half hour at 37° C.
5. Make several agar slants from the broth tube corresponding to the one giving best positive agglutination and typical appearance on Russell's medium. Incubate 24 hours at 37° C.
6. Add three-fourths c. c. of salt solution to each slant; scrape off growth, and collect the emulsion in large test tubes or Erlenmeyer flasks.
7. From each tube make a heavy smear and stain by the Gram method to determine purity of emulsion.

MEDIA, ETC.

1. Make 3 per cent agar +0.5, to phenolphthalein.
2. Sterilize 10 to 30 one-liter bottles with flat sides, plugged with absorbent cotton, by dry heat at 170° C. for 1½ hours.
3. Put 100 c. c. agar in each bottle, and sterilize in autoclave at 15 pounds for 1 hour.
4. Let agar harden over night with bottles flat. Invert and place in incubator on slight slant for 24 to 48 hours.
5. Make 0.85 per cent NaCl solution; autoclave at 15 pounds, 1½ hours. Plant 5 c. c. of the salt solution from each flask in freshly heated and cooled glucose broth fermentation tubes. Incubate 48 hours; discard if contaminated.
6. Make several absorbent-cotton swabs on wire rods; put in test tubes and sterilize by dry heat at 170° C. for 2 hours.

VACCINE.

1. Inspect each bottle for evidence of contamination, flame plug, and remove plug and flame mouth of bottle. Remove water of condensation with a sterile pipette.
2. Inoculate each bottle twice with well-charged swab from a tube of emulsion. Invert on slant and incubate 24 hours. Make plate cultures from emulsion which is left. (Inoculation may be done by placing a thin layer of the emulsion upon the agar and flowing it evenly over the surface.)

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[1] Bulletin 2, War Department, Office of the Surgeon General, p. 33, January, 1913.

3. Examine each bottle with great care and discard any bottles which have growths not typical of a typhoid culture or which show Gram positive organisms on staining. This is a most important step in the procedure, because if any spore-bearing organisms are passed over and get into the vaccine they will not be killed by subsequent measures and the vaccine will not be sterile.

4. Add 30 to 50 c. c. of salt solution to each bottle; place on slight slant, so that salt solution will moisten growth for few minutes. Wash off growth by holding the flask in the hands and slightly tilting the bottle from side to side so that the fluid flows across the surface of the agar. Collect the emulsion in 500 c. c. flasks.

5. Mix collections, measure emulsion, and take sample for counting.

6. Pour emulsion into 500 c. c. flasks, put tin foil on plug, weight with lead around neck and put in water bath. A control flask with salt solution and thermometer is put in bath. Another thermometer is suspended in the bath direct. The water should come above the fluid in the flasks by 1 to 2 inches.

7. Heat emulsion at 53° C. to 54° C. in well-agitated water bath, watched constantly to keep the temperature accurate. Remove flask after one hour at this temperature.

8. The neck of the flask should be thoroughly flamed to a point below the level heated by the water bath.

9. From the count estimate amount of salt solution to be used to give 1,000,000,000 bacilli per cubic centimeter and add trikresol to diluting fluid, so that the final strength of trikresol will be 0.4 per cent. Dilute vaccine according to count in large sterile bottles. Trikresol should be entirely in solution before adding to vaccine. Its solution is more prompt if added to warm salt solution and shaken during the addition.

10. After 12 to 24 hours at room temperature take sample for culture.

COUNTING.

1. A well mixed sample of the emulsion is placed in a small sterile conical glass.

2. A portion of this is diluted in a red-blood cell counting pipette using the following solution as a diluent: five per cent phenol solution, 100 c. c.; Ziehl-Nielsen carbol-fuchsin, 0.25 c. c. Mix and let stand 10 minutes and filter.

This diluting fluid kills the typhoid bacilli and stains them light red.

3. Count the number of bacteria in a Helber counting chamber in a manner similar to counting blood cells.

4. Estimate the number of bacteria per cubic millimeter of emulsion. (The Helber counting chamber is 0.02 mm. deep, the blood counting chamber 0.1 mm. Hence the capacity of the Helber chamber is one-fifth that of the blood counting chamber.)

TESTS.

1. Plate 2 loopfuls of emulsion left from inoculation on 2 sets of plates.

2. Plate 0. 1. c. c. heated vaccine in each of 3 plates. These plates should show some colonies of typhoid, but no others.

3. Plant cresolized vaccine in at least 6 anaerobic tubes, of freshly heated and cooled glucose broth, 5 drops in 3 tubes and 20 drops in 3 others. These tubes should be sterile. If not, the vaccine must be discarded. Incubate 7 days.

4. If above tests are satisfactory at end of 48 hours the following tests are proceeded with: Inoculate 2 guinea pigs, 1.5 c. c. intraperitoneally; 2 white mice, 0.5 c. c. subcutaneously. Give each of 3 rabbits, 0.5 c. c.; after 3 days 1 c. c.; after 6 days 1 c. c., all subcutaneously. Agglutination is tested after 5 days and should equal the controls.

Final agglutination should run at least to 1-500 dilution. The mice and the guinea pigs are used as a test for tetanus. The mice also serve as a check on the amount of preservative. The rabbits are used as a test for agglutinin production.

FILLING OF AMPULES.

1. Any apparatus that reduces the possibility of contamination to a minimum may be used.

2. Care must be taken to see that cracked ampules are excluded and that the seal is perfect. To do this, place the filled sealed ampules in a water bath at 40° C.; stir gently to insure uniform heating to this temperature. Remove the ampules to an iced water bath to which sufficient methylene blue is added to give it a deep blue color. After sufficient time has elapsed to allow all ampules to be cooled, remove the ampules and rinse in tap water. The contents of defective ampules will be colored blue.

TEST OF FINISHED PRODUCT.

1. At least 3 ampules, selected at random from each lot of the finished product are examined, or 3 per cent if more than 100 are filled at one time, up to 20 ampules for each lot. These are tested for sterility by planting from each ampule 5 drops in a Smith fermentation tube, and 20 drops in a similar tube, containing about 30 c. c. of recently heated and cooled glucose broth having a reaction of + 0.5 per cent to phenolphthalein at 100° C.

2. The tubes are inoculated and examined after two, four, and seven days; they should be agitated after each examination and the lowest part of the bend should be examined for slight growth before agitation.

3. If growth is found in any tube, a second test is made, using double the number of ampules used in the first test.

4. If the second test shows growth, the vaccine is discarded.

A copy of the form of record kept is shown here.

U. S. PUBLIC HEALTH SERVICE, HYGIENIC LABORATORY.

TYPHOID VACCINE.

Number 20. Date made, December 14, 1916. Amount, 10,800 c. c.

Time limit, June 14, 1917. Released, January 3, 1917.

CULTURE.

1916.

Dec. 11. Plated Rawling culture on Endo plates incubated at 37° 24 hrs.

Dec. 12. Fished 3 colonies from plates to Russell's tubes and duplicate broth tubes. Incubated at 37° 24 hrs. 3 typical; 0 atypical.

Dec. 13. Macroscopic agglutination test applied. 3 typical; 0 atypical. From selected culture planted 10 agar slants. Incubated 37° 24 hrs.

Dec. 14. Made emulsion for planting. 3 plates made from emulsion. Emulsion found pure by Gram stain.

Dec. 15. Plates show pure culture.

MEDIA AND SALT SOLUTION.

Dec. 11. 20 flasks agar (3 per cent) made by W. L.

Dec. 12. Agar placed in incubator at 37°.

Dec. 14. No growth in 19 flasks; growth in 1 flasks.

Dec. 11. 20 liters salt solution (.85 per cent) made by W. L. Sterilized 1½ hrs. Autoclave at 120° C.

Dec. 12. 5 c. c. salt solution planted in glucose broth from each of 20 flasks. Incubated 37° 24 hrs. 4.33 c. c. trikresol added to each liter.

Dec. 14. 20 flasks, sterile: Growth from 0 flasks.

VACCINE.

Dec. 14. 19 flasks inoculated twice with emulsion. Incubator, 37° at 11 a. m. 3 plates made from remaining emulsion.

Dec. 15. 1 flask rejected; accidental contamination; growth from 18 flasks washed off with salt solution. Amount of emulsion, 720 c. c.

Dec. 16. Plates made from planting emulsion show pure culture.

1916.
Dec. 15. Emulsion count, 15,000,000,000 per c. c.
Emulsion killed at 53°–54°. 1 hour. 3 plates made from killed emulsion (1. c. c. each).
Emulsion diluted with cresolized salt solution (0.4° trikresol).
Number of flasks, 10.
Quantity of emulsion, 720 c. c.
Quantity of salt solution used, 10,080 c. c.
Quantity of trikresol used, 43.3 c. c.
Quantity of vaccine, 10,800 c. c.
Dec. 16. 3 glucose broth fermentation tubes planted 5 drops to tube.
3 glucose broth fermentation tubes planted 20 drops to tube.
Dec. 18. 2 guinea pigs inoculated with 1.5 c. c. each intraperitoneally.
2 white mice inoculated with 0.5 c. c. subcutaneously.
3 rabbits inoculated with 0.5 c. c. subcutaneously.
Dec. 23. 3 rabbits inoculated with 1.0 c. c. subcutaneously.
Dec. 28. 3 rabbits inoculated with 1.0 c. c. subcutaneously.
1917.
Jan. 2. Rabbits bled from ear vein.
Jan. 3. Rabbits serum tested for agglutinins. Result; equal to control. I. A. B.
Dec. 18. Fermentation tubes show no growth. Dec. 23, no growth.
Dec. 23. Animals all living and well. Vaccine released. Jan. 3, 1917. Made by W. L.

FILLING OF AMPULES.

Vaccine No. 20. Lot A.

1917.
Jan. 5. 800 ampules (1 c. c.) filled by R. H. P. Sealed by R. H. P. 8 glucose broth fermentation tubes planted from siphon, incubated 37°.
34 ampules defective (methylene blue test) tubes planted from siphon, show no growth.

TEST OF FINISHED PRODUCT.

H. L. typhoid vaccine No. 20. Lot A.

Date labeled: June 14, 1917.
Date tested: January 6, 1917.
Packages: Glass ampules. Number tested, 20.
5 gtt. planted in 20 glucose broth fermentation tubes.
20 gtt. planted in 20 glucose broth fermentation tubes.
Incubated 37° January 6 to January 13, 1917.
1 c. c. injected subcutaneously into mouse. Phenol symptoms, negative.
Result of mouse test: Negative.
20 glucose fermentation tubes planted 5 gtt. Show: 19 no growth; 1 growth.
20 glucose fermentation tubes planted 20 gtt. Show: 20 no growth; 0 growth.
Tested by J. P. L.
Retest:
    40 glucose fermentation tubes planted 5 gtt. 40 show no growth.
    40 glucose fermentation tubes planted 20 gtt. 40 show no growth.

A COLORIMETRIC METHOD FOR THE ESTIMATION OF THE CRESOL OR PHENOL PRESERVATIVE IN SERUMS.[1]

By Elias Elvove, *Technical Assistant, U. S. Public Health Service.*

Most American manufacturers of therapeutic serums use cresol or the commercial "trikresol" as the preservative. The amount so added is most often about 0.4 per cent. Since, however, a precipitate usually forms on the addition of the cresol, the practice[2] of many manufacturers has been to add the cresol before the final filtration. This, of course, means that the product thus obtained may not have enough cresol remaining to act efficiently as preservative and also that the amount which remains may vary considerably. In order to determinine the effect of any given procedure on the cresol and also to check possible errors, it appears desirable to be able to estimate the amount of cresol in such serums by a comparatively simple method, such as might conveniently be used by the manufacturer himself for controlling his own product.

The literature examined indicated that there was no published work bearing directly on this subject. The nearest approach which was found in the literature is the determination of cresol in urine. According to Siegfried and Zimmermann,[3] this requires three preliminary separations. The first and third separations involve the distillation of a comparatively large volume with the aid of steam, while the second separation requires a similar distillation which is accompanied also by the passage of a current of carbon dioxide. The solution thus obtained is finally titrated by the method described by these authors. Such a procedure can hardly be regarded as simple enough for the purpose in view and also involves much time and labor.

In carrying out their method, Siegfried and Zimmermann use Millon reagent for testing the completion of the distillation of the cresol by applying this reagent to a small portion of the last distillates. It, therefore, occurred to the writer that this qualitative test might be worked out as a colorimetric procedure to estimate small amounts of cresol. After some experiments along this line

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[1] Manuscript submitted for publication July 5, 1916.

[2] The writer is indebted to Dr. J. P. Leake, of this laboratory, for this information.

[3] Biochem. Zeit., 34, 462–472 (1911).

had already been carried out, it was found that Bach [1] had actually applied a similar method for the determination of phenols in sewage. Inasmuch, however, as the procedure worked out for the present case differs from Bach's procedure in some respects, which makes it simpler and more sensitive, it is interesting to note these points of difference.

According to Bach, one of the probable reasons why Millon reagent had not previously been applied for the quantitative estimation of phenols is that the reaction becomes intense only on heating and that the colorations thus produced are not always the same. He, therefore, tries to overcome this difficulty by standardizing the manner of heating, directing that the heating be carried just to boiling.[2] In the course of the experiments which the writer carried out it was found, however, that the heating may be entirely omitted and that the color can be brought out in a comparatively short time even at ordinary temperature by simply increasing the amount[3] of Millon reagent. Furthermore, this manner of carrying out the test serves also to render it more sensitive. Thus Bach [4] states that by his method 0.05 mgm. phenol in 10 c. c. or 1 in 200,000 does not yield any coloration, whereas by the writer's procedure this same amount of phenol in the same volume of solution was found to give a decided reddish color on mixing with 5 c. c. of the Millon reagent and allowing to stand 30 minutes, although this really makes the final concentration 1 in 300,000, and even half of that amount in 10 c. c. when similarly treated could be readily distinguished from the corresponding control.

Since some of the therapeutic serums are comparatively expensive, it appeared desirable to make it possible to use only a comparatively small amount of the serum for the determination. After some preliminary experiments, the following procedure was adopted: Five-tenths c. c. of the sample measured out [5] with a finely graduated and accurately standardized 1 c. c. pipette was transferred into a 1,000 c. c. Erlenmeyer flask, diluted with distilled water to 275 c. c. and then mixed with 25 c. c. of diluted sulphuric acid (H_2SO_4 of specific gravity 1.84, mixed with an equal volume of distilled water). The flask was connected with a suitable glass condenser and the contents distilled until 200 c. c. were collected in a 200 c. c. measuring flask. The distillate was filtered [6] through a dry folded filter into a glass-stoppered bottle and then thoroughly mixed. To 5 c. c. of the freshly prepared Millon reagent,[1] in a narrow [2] 50 c. c. Nessler tube, there were added 10 c. c. of that distillate and the whole mixed thoroughly with the aid of a bulbed glass rod. Simultaneously [3] 10 c. c. from each of four standard trikresol solutions were similarly mixed with half their volume of the Millon reagent. The color which developed in the sample, after it had stood 10 minutes, was compared with the colors developed by the standards. These standards were so prepared that the colors yielded by them would be matched by that of the sample when treated as just described if the latter contained in the original undiluted preparation 0.1, 0.2, 0.3, or 0.4 per cent of trikresol, respectively. Since the amount of trikresol originally added is about 0.4 per cent and the only fact to be determined in most cases is how much of this may have been lost in the precipitate, the color produced by the sample must come within the range of these standards. Having thus determined which of these standards comes nearest to matching the color produced by the sample, that standard was then used for making a closer reading by transferring [4] into a small measuring cylinder a sufficient amount of the deeper solution to make the remainder in the Nessler tube match the weaker solution and calculating the value of the unknown from the proportion of the heights of the two columns of liquid.

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[1] Zeit. anal. Chem., *50*, 736–740 (1911).

[2] According to Bach, some practice will enable one to carry out the heating over a free flame just to boiling without losing any liquid by spirting and so that the loss by evaporation will be in all subsequent cases practically the same and negligible.

[3] Bach uses 0.2 c. c. of the Millon reagent for 10 c. c. of phenol solution.

[4] Loc. cit.

[5] In case of very viscid samples it is preferable either to use a capacity pipette, washing it out with some of the diluent, or to weigh the sample.

[6] The object of the filtering was to remove a slight scum which often formed on the surface.

In order to determine whether this procedure effects the complete recovery of the cresol in the distillate or, if not, what proportion is thus recovered, the following experiments were carried out:

To 0.5 c. c. portions of untreated horse serum were added known amounts of dilute trikresol solution so as to correspond to the amounts which would be present if the serum contained 0.1, 0.2, 0.3, or 0.4 per cent trikresol, respectively, and the above procedure was applied. The results obtained by comparison with corresponding trikresol solutions indicated a complete recovery of the trikresol, but the presence of the serum in the original mixture appears to increase very slightly the depth of color obtained on mixing with the Millon reagent.

On applying the Millon reagent to a distillate which was similarly obtained from a mixture to which the serum alone, without trikresol, was added, only a very slight coloration could be observed on comparing with a corresponding control with distilled water and none whatever in comparing with the lowest of the above standards.

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[1] Prepared as described in the subsequent part of this paper.

[2] About 15 mm. internal diameter.

[3] Since the color changes on standing, it was aimed to treat the sample and the standards with the Millon reagent as nearly simultaneously as possible. The Millon reagent was added first to all the tubes, being careful not to get any of the reagent on the walls. The sample and standards, respectively, were then added quickly, running the solutions on the sides of the tubes and avoiding mixing with the reagent. The mixing was then effected in all the tubes nearly simultaneously with the aid of bulbed glass rods.

[4] This procedure was used when there was only a very slight difference between the sample and one of these primary standards. When there was considerable difference, this preliminary comparison served only to indicate the dilutions which would give a closer match, and the treatment with the Millon reagent was then repeated on these. When, however, several determinations were carried out simultaneously, the samples themselves very often supplied advantageous intermediary colors, which were often utilized as intermediary standards.

It appears preferable, therefore, to prepare each of the above standards by mixing the proper amount of trikresol solution [1] with 0.5 c. c. of the serum and proceeding as in the actual determination, using the 200 c. c. distillate thus obtained as the corresponding standard trikresol[2] solution.

In looking up the procedure for the preparation of the Millon reagent, it was noted that there was some disagreement between different authors. The chief difference appears to relate to the proportion of nitric acid to be used for dissolving the mercury. Thus, according to Abderhalden,[3] Neuberg,[4] Bloxam,[5] Simon,[6] and Wood,[7] 1 part of mercury is to be dissolved in 2 parts by weight of concentrated [8] nitric acid, while, according to Allen,[9] Neubauer and Vogel,[10] Schmidt,[11] and Tyson,[12] 1 part of mercury is to be dissolved in only 1 part by weight of the concentrated nitric acid. Sadtler and Coblentz in one [13] place in their book direct that 1 part of the mercury be dissolved in 1 part of the concentrated nitric acid while in a later portion of the same book [14] they direct that 2 parts of nitric acid be used. That the proportion of nitric acid used is not a point which may be overlooked, since the resulting reagents act differently, may be seen from the following experiment. Millon reagent was prepared according to both of the above nethods, i. e., in one case (No. 1) the mercury was dissolved in an approximately [15] equal weight of concentrated [16] nitric acid and in the other case (No. 2) in twice its weight of the same nitric acid. In neither case was any extraneous heat applied. In both cases, the solution of the mercury in the nitric acid was diluted with twice its volume of distilled water. To 5 c. c. of each of these reagents there were added 10 c. c. of a 0.001 per cent trikresol solution. After mixing and allowing to stand 10 minutes at ordinary temperature, it was found that whereas the solution which had been treated with reagent No. 1 had a decided red color, that which was treated with reagent No. 2 appeared in comparison almost colorless. In the work reported in this paper, therefore, reagent No. 1 was used exclusively.

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[1] The stock solution for the preparation of this solution was about 1.6 per cent (prepared by weighing out about 1.6 gm. of the trikresol and diluting to 100 c. c. with distilled water). A 1/200 per cent trikresol solution is convenient for this purpose. Ten, 20, 30, and 40 c. c. of this solution would correspond to 0.1, 0.2, 0.3, and 0.4 per cent trikresol, respectively, when operating as herein described.

[2] Of course, other cresol or phenol standards can be prepared in a similar manner.

[3] Abderhalden: Handbuch der Biochemischen Arbeitsmethoden, 1910, vol. 2, p. 351.

[4] Neuberg: Der Harn, 1911, Pt. I, p. 470.

[5] Bloxam's Chemistry, 8th ed., p. 736.

[6] Simon: Clinical Diagnosis, 4th ed., p. 425.

[7] Wood: Chemical and Microscopical Diagnosis, 1905, p. 689.

[8] The concentrated nitric acid is in most cases described as having a specific gravity of 1.40 or 1.42.

[9] Allen: Commercial Organic Analysis, 2d ed., Vol. IV, p. 20.

[10] Neubauer and Vogel: Analyse des Harns, 10th ed., p. 153.

[11] Schmidt: Lehrbuch der Pharmaceutischen Chemie, 4th ed., vol. 1, p. 1010.

[12] Tyson: Practical examination of the urine. 9th ed., p. 62.

[13] Sadtler and Coblentz: Pharmaceutical and medical chemistry, 3d ed., vol. 1, p. 497.

[14] Ibid., p. 852.

[15] In preparing the Millon reagent by dissolving mercury in an equal weight of concentrated nitric acid, Allen (loc. cit.) suggests the use of the proportion of 1 c. c. mercury to 10 c. c. concentrated nitric acid. This proportion was used in this work but the required amount of mercury was determined by weight instead of volume, using the ratio of 13.6 gm. for 1 c. c. In diluting the mercury solution with twice its volume of distilled water, the latter was added in several portions and mixed well after each addition. A small amount (2 per cent by volume) of the concentrated nitric acid was then added, which on mixing redissolved whatever precipitate formed on the dilution with the water. On the basis of these proportions, the Millon reagent used in this work was prepared as follows: Added 50 c. c. concentrated nitric acid (specific gravity 1.405 at 25° C.) to 68 gms. mercury in a 250 c. c. beaker. The resulting solution usually measured 46 c. c. and therefore was diluted with 92 c. c. distilled water. To this there were then added 2.76 c. c. of the concentrated nitric acid and mixed until the precipitate, which often formed, completely redissolved.

[16] The specific gravity of this acid was 1.405 at 25° C.

After it was found that the usual heating with the Millon reagent may be omitted and that the color can be brought out in a comparatively short time even at ordinary temperature by simply increasing the proportion of the Millon reagent, it was thought desirable to try a series of experiments with varying proportions of the Millon reagent in order to determine which proportion would be the most suitable. As the test solution for these experiments, a 0.001 per cent trikresol solution was used. The results obtained are given in Table I.

TABLE I.—*Effect of varying the proportion of the Millon reagent on the resulting color with trikresol.*

[Time, 10 minutes.]

No. of experiment.	Amount of trikresol solution (c. c.).	Amount of Millon reagent (c. c.).	Interference by formation of precipitate.	Appearance of mixture.
1	10	0.1	Yes	Almost colorless.
2	10	.5	...do	Slightly yellowish.
3	10	1.0	...do	Red.[1]
4	10	3.0	Occasionally, very slight.	Decidedly red.
5	10	5.0	No	Do.
6	10	7.0	...do	Decidedly red, with slight shade of yellow.
7	10	10.0	...do	More yellowish than No. 6.
8	10	15.0	...do	Decidedly yellowish.

[1] This solution and the preceding developed very decided red colors on longer standing, but since more time is required and on account of the interference by the formation of a precipitate, these proportions of the Millon reagent are not well suited in practice.

The results given in Table I show that the best results were obtained when the proportion of the Millon reagent was 3 or 5 c. c. to 10 c. c. of the trikresol solution. When the proportion of the Millon reagent was increased to 10 or 15 c. c. for each 10 c. c. of trikresol solution, the characteristic red color was no longer obtained. As between the use of 3 or 5 c. c. of the Millon reagent for each 10 c. c. of the trikresol solution, the latter appears preferable, since with this proportion of the Millon reagent there is apparently less tendency to form a precipitate, which was observed to form occasionally to a slight extent on standing when only 3 c. c. of the Millon reagent was used.

It was also found that even the more sensitive reagent, namely, that prepared by dissolving the mercury in approximately its equal weight of concentrated nitric acid, loses its sensitiveness if it is afterwards mixed with an excess of nitric acid. In order, therefore, to determine the safe limits, a series of experiments was carried out with varying amounts of nitric acid, using the same test solution as in the previous experiments, namely, a 0.001 per cent trikresol solution. The results obtained are given in Table II.

TABLE II.—*Effect of varying the proportion of nitric acid on the resulting color with trikresol.*

[Time, 10 minutes.]

No. of experiment.	Amount of trikresol solution (c. c.).	Amount of Millon reagent (c. c.).	Amount of additional nitric acid (c. c.).[1]	Appearance of the Millon reagent+HNO_3 mixture.	Appearance of the Millon reagent + HNO_3 + trikresol mixture.
1	10	5	None.		Decidedly red.
2	10	5	0.1	Very slightly yellowish	Do.
3	10	5	.5	do	Very slightly red.
4	10	5	1.0	Slightly yellowish	Slightly yellowish.
5	10	5	3.0	Slightly greenish yellow	Slightly greenish yellow.
6	10	5	5.0	Decidedly greenish yellow	Do.
7	10	5	7.0	Decidedly greenish yellow, but deeper than No. 6.	Greenish yellow.
8	10	5	10.0	Decidedly greenish yellow, but deeper than No. 7.	Greenish yellow, but deeper than No. 7.

[1] The specific gravity of the nitric acid was 1.405 at 25° C.

Table II shows that when even as little as 0.5 c. c. of concentrated nitric acid was added to 5 c. c. of the Millon reagent (experiment No. 3) the inhibiting effect on the test was very decided, and when 5 c. c. of the Millon reagent was mixed with 1 c. c. of the concentrated nitric acid (experiment No. 4) the inhibiting effect on the characteristic red color was practically complete.

While most of the therapeutic serums at present on the market contain cresol as the preservative, there appear to be a few which contain phenol. In order to see whether or not the behavior of phenol with varying proportions of the Millon reagent and with Millon reagent which had been mixed with varying amounts of nitric acid was similar to that observed in the case of trikresol, similar experiments were carried out with phenol. As in the case of the trikresol, the concentration of the test solution was 0.001 per cent. The results obtained with varying proportions of Millon reagent are given in Table III.

TABLE III.—*Effect of varying the proportion of the Millon reagent on the resulting color with phenol.*

[Time, 30 minutes.[1]]

No. of experiment.	Amount of trikresol solution (c. c.).	Amount of Millon reagent (c. c.).	Interference by formation of precipitate.	Appearance of mixture.
1	10	0.1	Yes	Almost colorless.
2	10	.5	do	Slightly yellowish.
3	10	1.0	do	Faintly red.
4	10	3.0	Occasionally, very slight.	Decidedly red.
5	10	5.0	No	Do.
6	10	7.0	do	Decidedly red with slight shade of yellow.
7	10	10.0	do	More yellowish[2] than No. 6.
8	10	15.0	do	More yellowish[2] than No. 7.

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[1] The reason for adopting a longer period in this case was because it was found that the color developed more slowly than with trikresol.

[2] On longer standing, these yellowish colors were displaced by red colors.

It is seen (Table III) that the effect of varying the proportion of the Millon reagent in the case of phenol is similar to that observed in the case of trikresol (Table I).

The results obtained in the case of the phenol with Millon reagent which had been treated with varying amounts of nitric acid are given in Table IV.

TABLE IV.—*Effect of varying the proportion of the nitric acid on the resulting color with phenol.*

[Time, 30 minutes.]

No. of experiment.	Amount of phenol solution (c. c.).	Amount of Millon reagent (c. c.).	Amount of additional[1] nitric acid (c. c.).	Appearance of mixture.
1	10	5	None.	Decidedly red.
2	10	5	0.1	Do.
3	10	5	.5	Very slightly red.
4	10	5	1.0	Slightly yellowish.
5	10	5	3.0	Slightly greenish yellow.
6	10	5	5.0	Do.
7	10	5	7.0	Greenish yellow.
8	10	5	10.0	Greenish yellow, but deeper than No. 7.

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[1] The usual procedure of adding the test solution last was likewise followed here, the additional nitric acid being mixed in the Nessler tube with the 5 c. c. Millon reagent shortly before the introduction of the test solution.

The effect of varying the amount of nitric acid in the case of phenol is similar to that observed in the case of the trikresol.

The following results (Table V) were obtained by the procedure herein described with mixtures of serum and trikresol and of serum and phenol, the amounts of trikresol or phenol in which were unknown to the writer at the time the work was done, having been submitted to the writer as "unknowns" by Dr. J. P. Leake of this laboratory.

TABLE V.—*Results with mixtures of serum and trikresol and of serum and phenol.*

No. of mixture.	Preservative.	Percentage of preservative (given).	Percentage of preservative (found).	Error.
1	Trikresol	0.24	0.25	+0.01
2	do	.38	.38	.00
3	do	.46	.46	.00
4	Phenol	.23	.22	− .01
5	do	.43	.41	− .02

The procedure described in this paper was also applied in the determination of the cresol preservative in 38 samples of therapeutic serums which were obtained from several American manufacturers. The results obtained are given in Table VI.

TABLE VI.—*Determination of the cresol preservative in some commercial serums.*

No. of sample.	Name of serum.	Manufacturer.	Percentage of cresol.	No. of sample.	Name of serum.	Manufacturer.	Percentage of cresol.
1	Tetanus antitoxin	A	0.32	20	Tetanus antitoxin	C	0.44
2	do	B	.17	21	do	C	.44
3	do	B	.18	22	do	D	.33
4	do	B	.18	23	do	E	.46
5	do	B	.22	24	Diphtheria antitoxin	C	.30
6	do	B	.24	25	do	C	.34
7	do	B	.28	26	Antidysenteric serum	C	.31
8	do	B	.28	27	do	C	.42
9	do	B	.28	28	do	C	.51
10	do	B	.34	29	Antistreptococcic serum	C	.40
11	do	C	.32	30	do	C	.43
12	do	C	.33	31	do	C	.49
13	do	C	.34	32	Antimeningitis serum	C	.20
14	do	C	.35	33	do	C	.26
15	do	C	.35	34	do	C	.26
16	do	C	.40	35	do	C	.26
17	do	C	.42	36	do	C	.30
18	do	C	.42	37	do	C	.40
19	do	C	.43	38	do	C	.42

These results are based mostly on the Millon color value obtained for the cresol used by manufacturer B, which was known to have been used also by manufacturer C. A sample of the cresol used by manufacturer A was found to yield practically the same Millon color value as that obtained with the cresol from manufacturer B. Another sample of commercial cresol, however, which has recently been placed on the market as a serum preservative was found to give a very decidedly lower Millon color value than the above when the test was carried out as herein described for cresol. Its behavior with the Millon reagent indicated that it contained some phenol. This shows, therefore, that when dealing with the commercial cresols, in order to obtain reliable quantitative results it is necessary either to prepare the standards directly from the same lot of cresol which was used for the preservative or to determine the relative Millon color value between it and that which is used for the preparation of the standards. Manufacturers D and E used the commercial trikresol; hence in their cases the results are based on the Millon color value obtained with a sample of trikresol. The difference between the Millon color value of the latter and that of the cresol from manufacturer B was found to be about 7 per cent, the trikresol giving the deeper color.

The results given in Table VI show also that there was quite a variation in the amounts of preservative in the different samples of even the same class of serum from the same manufacturer. Thus the highest amount found (No. 10) in the case of tetanus antitoxin from manufacturer B was double that of the lowest (No. 2). Similarly, the highest amount found (No. 38) in the case of antimeningitis serum from manufacturer C was more than double that of the lowest (No. 32). On comparing the results obtained in the case of tetanus antitoxin from manufacturers B and C, we see that on the whole those from B contained decidedly less preservative than those from C. The average of the 9 samples examined from manufacturer B was 0.24 per cent, while the average of the 11 samples examined from manufacturer C was 0.38 per cent. These results, therefore, confirm the opinion expressed in the first part of this paper of the desirability of better controlling the amount of preservative in such serums and thus insuring a more uniform product. It is believed that the method herein proposed answers this want, especially since it is comparatively easy to carry out and enables the manufacturer to control his own product with only little additional work or expense.

TOXICITY OF CERTAIN PRESERVATIVES USED IN SERUMS, VIRUSES, AND VACCINES.[1]

By JAMES P. LEAKE, *Passed Assistant Surgeon*, and HUGH B. CORBITT, *Sanitary Bacteriologist, U. S. Public Health Service.*

INTRODUCTION.

The following experiments were undertaken to determine the toxicity of certain disinfectants used in biologic products and to check the experiments of Hale (Bull. 88, Hyg. Lab., Wash., 1913), who determined a minimum lethal dose of phenol and trikresol. Certain variables appeared to be capable of influencing the accuracy of the results, so that the data obtained were examined to estimate the importance of these factors. These are discussed under dilutions, relative weight susceptibility, and relative sex susceptibility.

METHODS.

In general the methods used were those recommended by Hale in the above-mentioned bulletin. The solutions were made by weight and diluted to the required volume in distilled water. The amounts injected were measured in the syringes used, which were of Luer type, 2 c. c. graduated to 0.1 c. c., or in some experiments 1 c. c. graduated to 0.01 c. c.

The calculations of dosage were made in the following manner: The mice were marked, placed four in a jar, and weighed with torsion balance before feeding on the morning of inoculation. The amount to be injected was found by multiplying the weight of the mouse by the intended dose in grams of disinfectant per gram body weight and dividing by the grams of disinfectant per c. c. of solution.

Amount to be injected =

$$\frac{\text{Weight of mouse} \times \text{intended dose in grams per gram body weight}}{\text{Grams of substance per c. c. of solution.}}$$

This volume, measured to the nearest 0.1 or 0.05 c. c., was injected subcutaneously. From the actual amount of the solution injected was calculated the actual dosage received per gram weight of mouse:

Actual grams per gram body weight =

$$\frac{\text{Volume of solution injected} \times \text{grams of substance per c. c. of solution}}{\text{Weight of mouse.}}$$

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[1] Manuscript submitted for publication Jan. 5, 1917.

The record of data for each experiment was kept in the following form:

No. of mouse.	Volume injected.	Volume intended.	Intended dosage per gram body weight.	Actual dosage per gram body weight.	Sex.	Symptoms.	Results.	Remarks.

Six of the 2 c. c. syringes used were tested and found to vary 0.1 to 0.01 c. c. from the stated volume. The average error was 0.045 c. c. This takes into account all variations except occasional slight leakage from the mouse after injection. All instances of visible leakage were rejected in the computation.

The substances used were phenol, trikresol, other cresols similar to trikresol, and glycerine. The stock phenol was made from pure crystalline phenol and titrated by the Division of Chemistry, Hygienic Laboratory, and further dilutions were prepared as needed. Trikresol (Schering), specific gravity 1.0388 at 20° C., was used for trikresol solutions. Glycerine solutions were prepared from Price's glycerine.

TOXICITY OF PHENOL IN DISTILLED WATER.

The object of the toxicity experiment was to determine the minimum lethal dose of the disinfectant used. Four mice were used on each dose, and four doses spaced at intervals of 10–15–20 per cent were given in each experiment. It was finally believed that 20 per cent intervals represented the smallest spacing consistent with accuracy.

The accompanying Table 1 shows the number of mice used on each dosage and the percentages of deaths.

TABLE 1.—*Toxicity of phenol in distilled water.*

Dosage in grams per gram mouse.	Mice injected.	Per cent of deaths.
0.000221 to 0.00027	45	31
0.000271 to 0.00033	57	61
0.000331 to 0.00041	67	85
0.000411 to 0.0005	41	93

It will be noted from this table that there is no definite MLD. Within the wide range of 0.0002 gram (the highest nonfatal dose) and 0.0005 gram—an increase of 150 per cent—there seems to be no fixed amount that will always kill 100 per cent of the mice used. If we assume the MLD to be that amount which will kill 80 per cent of he mice inoculated, the above results indicate a value between .00033 gram and 0.00041 gram, approximately 0.0037 gram phenol er gram weight of mouse.

Therefore in determining the toxicity coefficient of a coal-tar disnfectant it would seem necessary to use at least five mice on each lose.

TOXICITY OF TRIKRESOL.

Table 2 is a summary of the results of experiments with trikresol (Schering). These figures give the same conclusions as the phenol xperiment. In so far as the accuracy of the above tests permit, it s inferred that trikresol is as toxic as phenol, the MLD for 80 per ent of the mice falling between 0.00033 gram and 0.00041 gram per gram body weight of mice.

Table 2.—*Toxicity of trikresol in distilled water.*

Dosage in grams per gram mouse.	Mice injected.	Per cent of deaths
0.00018 to 0.00022	19	0
0.000221 to 0.00027	38	47
0.000271 to 0.00033	61	69
0.000331 to 0.00041	79	91
0.000411 to 0.0005	20	100
0.000501 to 0.00061	7	100

TOXICITY OF TRIKRESOL AND PHENOL IN NORMAL HORSE SERUM.

In this experiment two methods of dilution for trikresol were employed. (1) The trikresol was added directly to the serum drop by drop; a white flocculent precipitate resulted; the solution was shaken immediately before injection so that a proportionate amount of flocculi accompanied each dose. (2) A stock solution, 1.6 per cent of trikresol in water, was added to horse serum in sufficient amounts to give 0.4 per cent; this method gave a fairly clear solution. No differences in toxicity were noted in the two methods of adding trikresol, nor between the phenol and trikresol dilutions, though the number of mice on each was too small to draw conclusions.

The results of this experiment appear in Table 3. It is seen by comparing with Tables 1 and 2 that the use of serum does not seem to affect the toxicity of the disinfectants used here.

Table 3.—*Toxicity of phenol and trikresol in horse serum.*

Dosage in grams per gram mouse.	5 per cent phenol in serum.		4 per cent trikresol in serum.		Total.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
0.000221 to 0.00027	4	25	8	38	12	33
0.000271 to 0.00033	8	62	16	69	24	67
0.000331 to 0.00041	7	100	14	86	21	90
0.000411 to 0.0005	9	78	14	93	23	87
0.000501 to 0.00061	4	100	9	100	13	100

TOXICITY OF VARIOUS COMMERCIAL CRESOLS.

Cresols (similar to trikresol) from four biological laboratories—W, X, Y, and Z, and trikresol (Schering) from a frequently opened bottle at the Hygienic Laboratory, were used in the test. Samples W, X, and Z, though obtained from different sources, were made by the same chemical firm. The Schering trikresol is designated "O." Results are given in Table 4.

TABLE 4.—*Toxicity of soluble cresols in distilled water.*

Dosage, grams per gram mouse.	Cresol W.		Cresol X.		Cresol Y.		Cresol Z.		Cresol O.		Total.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
0.00018 to 0.00022	4	0	4	0	......	......	......	......	2	0	10	0
0.000221 to 0.00027	7	0	7	14	3	67	......	......	8	50	25	28
0.000271 to 0.00033	8	50	7	57	9	100	7	14	7	100	38	66
0.000331 to 0.00041	8	50	7	57	8	88	9	78	9	89	41	73
0.000411 to 0.0005	4	100	2	100	8	100	8	100	5	100	27	100
0.000501 to 0.00061	......	......	......	0	4	100	8	88	......	......	12	92

While the number of mice used is too small to draw definite conclusions, it would appear that the toxicity of cresols W, X, and Z are slightly less than phenol, and the toxicity of Y and O practically the same as phenol.

TOXICITY OF GLYCERINE.

The purpose of this experiment was to determine the minimum lethal dose of glycerine for white mice. The solutions were made from Price's glycerine weighed and diluted with distilled water to give solutions of 66⅔ per cent, 50 per cent, 25 per cent, and 20 per cent. In the measurement of the two concentrated solutions, 1 c. c. tuberculin syringes were used. The results are given in Table 5.

TABLE 5.—*Toxicity of glycerine.*

Dosage in grams per gram mouse	Glycerine in 20 and 25 per cent solutions.		Glycerine in 50 and 66⅔ per cent solutions.		Total.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
0.0075 to 0.009	39	13	8	12	47	13
0.0091 to 0.011	73	66	8	63	81	65
0.0111 to 0.0135	46	85	8	75	54	83
0.01351 to 0.0165	16	88	8	100	24	92

Here, as with phenol and phenol-like disinfectants, the MLD is not a fixed value. About 80 per cent died on doses between 0.011 and 0.0135 gram, so that if we take an 80 per cent fatality as determining the MLD, that quantity will be approximately 0.012 gram of glycerine per gram body weight of mouse.

TOXICITY OF GLYCERINE-PHENOL AND GLYCERINE–TRIKRESOL MIXTURES.

TABLE 6.—*Toxicity of glycerine-phenol and glycerine-trikresol mixtures.*

Dosage in grams of glycerine per gram mouse.	25 per cent glycerine–0.5 per cent phenol.		50 per cent glycerine–0.25 per cent phenol.		20 per cent glycerine–0.4 per cent trikresol.		66⅔ per cent glycerine–0.4 per cent trikresol.		Total.	
	Mouse injected.	Per cent of deaths.	Mouse injected.	Per cent of deaths.	Mouse injected.	Per cent of deaths.	Mouse injected.	Per cent of deaths.	Mouse injected.	Per cent of deaths.
0.0029 to 0.0035	4	0							4	0
0.00351 to 0.0043	4	0							4	0
0.00431 to 0.0052	7	0							7	0
0.00521 to 0.0064	8	0	4	0					12	0
0.00641 to 0.0078	4	0	4	25	4	0			12	8
0.00781 to 0.0095	8	25	7	57	5	20	4	50	24	38
0.00951 to 0.0116	8	100	8	100	3	67	4	50	23	87
0.01161 to 0.01419			5	100	4	100	4	100	13	100
0.0142 to 0.0172	4	100	4	100			4	100	12	100

Table 6 shows the proportions of the glycerine-phenol and glycerine-trikresol mixtures and the results of the tests thereon. From the other toxicity experiments it is concluded that more mice than were available should have been used on these mixtures. However, it will be seen that the approximate MLD seems to be slightly smaller than that of pure glycerine.

The mice inoculated with the 50 and 66⅔ per cent solutions of glycerine with smaller amounts of coal-tar disinfectants gave glycerine symptoms. The mice inoculated with 20 and 25 per cent glycerine-coal-tar disinfectant mixtures gave phenol symptoms.

BROADNESS OF RANGE OF PARTIAL TOXICITY.

In chart 1 a curve is plotted to show the percentage of mice dying on each dose of phenol or trikresol; chart 2 gives the same data for pure glycerine solutions. Each group of doses—0.00018 to 0.00022, 0.000221 to 0.00027, etc., is plotted at the median point of the group. The similarity of these curves is apparent, also the broadness of their slope, extending through a range greater than that between zero dosage and the beginning of fatal toxicity. Both curves slope off even more gradually beyond 80 per cent mortality, so that this point has been taken as the most practical criterion for the "minimum lethal dose" as commonly understood. The dose determinable with the greatest accuracy, however, would be that represented by the steepest part of the curve, possibly the dose giving 33 per cent mortality.

TIME OF DEATH.

Following Hale's bulletin, all mice living over 24 hours were considered to have survived the dose. Two definite periods of death were noted in mice inoculated with coal-tar disinfectants. The first period was between 15 and 60 minutes, when one-sixth to one-third of the deaths occurred. The second period was between 1 hour 30 minutes and 4 hours, when practically all of the remainder of deaths occurred.

CHART 1.- MORTALITY ON DIFFERENT DOSES OF PHENOL AND CRESOL. 683 MICE.

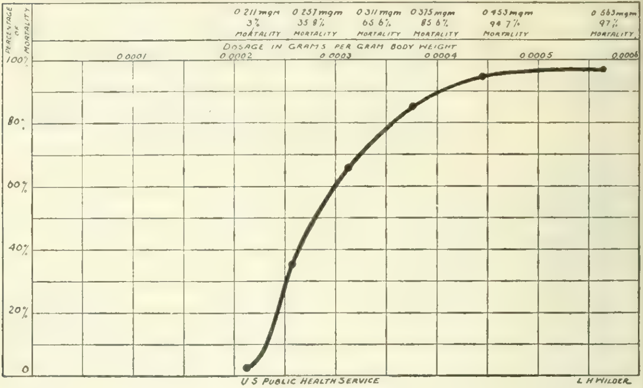
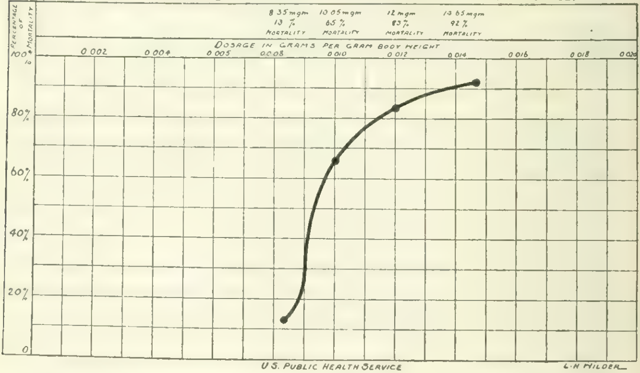


CHART 2:- MORTALITY ON DIFFERENT DOSES OF GLYCERINE. 206 MICE.



In the use of glycerine a different death period is found. Most of the mice died between 30 and 90 minutes. The average time seemed to be about 1 hour.

The mice injected with phenol-like substances invariably showed the tremors characteristic of this poison, sometimes with paralytic depression just before death. The symptoms with glycerine poisoning are also spastic in part, but more convulsive, the mice often leaping suddenly with contractions of the leg muscles; the final stage of glycerine poisoning was always profound depression. The glycerine symptoms are not seen until just before death, while the phenol symptoms appear within 10 minutes after injection. Hemoglobinuria was more frequently noted with glycerine than with phenol and cresol.

DILUTION OF DISINFECTANT.

In order to test the effect of dilution upon the toxicity of a substance, solutions of different strength were used. These dilutions and results appear in Tables 7 and 8 and 5. Considering Tables 8 and 5, no difference in toxicity is apparent. In Table 7, however, the use of a more dilute solution seems to give a larger percentage of deaths throughout than does the 1 per cent solution.

Therefore from the above it appears that phenol is slightly more toxic as the solutions are made more dilute from 1 to 0.25 per cent.

TABLE 7.—*Phenol in distilled water, various dilutions.*

Dosage in grams per gram mouse.	1 per cent.		0.5 per cent.		0.25 per cent.		Total.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
0.000221 to 0.00027....	14	14	27	37	4	50	45	31
0.000271 to 0.00033....	25	52	28	68	4	75	57	61
0.000331 to 0.00041....	34	80	30	90	3	100	67	85
0.000411 to 0.00050....	13	92	24	92	4	100	41	93

TABLE 8.—*Trikresol in distilled water, various dilutions.*

Dosage in grams per gram mouse.	1 per cent.		0.5 per cent.		0.4 per cent.		0.3 per cent.		Total.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
0.00018 to 0.00022....	3	0	2	0	7	0	7	0	19	0
0.000221 to 0.00027...	13	46	6	33	11	63	8	36	38	47
0.000271 to 0.00033...	26	77	4	50	23	61	8	75	61	69
0.000331 to 0.00041...	39	92	6	100	24	88	10	90	79	91
0.000411 to 0.00050...	11	100	1	100	6	100	2	100	20	100
0.000501 to 0.00061...	2	100	3	100	.....	.....	2	100	7	100

RELATIVE WEIGHT SUSCEPTIBILITY.

The following study was made to determine whether mice of different weights were equally susceptible to the same amount of disinfectant per gram body weight. After being weighed the mice were arranged according to weight and then given a serial number. The arrangement by weight consisted in placing the heaviest mouse of a series of 16 first, the lightest second, and alternately heavy and light. This gave 4 mice on a given dose—2 light and 2 heavy. Four different doses of each solution were given as one experiment.

In tabulating the results the mice were grouped according to dose and arranged in five columns according to weight. (See Tables 9 and 10.)

TABLE 9. —*Weight fatalities in mice inoculated with phenol and trikresol* (*679 mice*).

Dosage in grams per gram mouse.	12 to 15 gm.		15.01 to 18 gm.		18.01 to 21 gm.		21.01 to 24 gm.		24.01 to 27 gm.		Total.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
Aqueous phenol solutions.												
0.000221-0.00027	3	0	16	31	13	38	7	29	6	33	45	31
0.000271-0.00033	3	100	17	53	24	58	13	69	......	......	57	61
0.000331-0.00041	......	......	19	90	35	86	13	77	......	......	67	85
0.000411-0.0005	......	......	15	93	18	94	7	86	1	100	41	94
Aqueous trikresol solutions.												
0.00018-0.00022	2	0	5	0	8	0	2	0	2	0	19	0
0.000221-0.00027	......	......	13	54	15	40	7	71	3	0	38	47
0.000271-0.00033	1	0	21	67	26	81	10	40	3	100	61	69
0.000331-0.00041	1	100	17	94	42	90	19	90	......	......	79	91
0.000411-0.0005	......	......	7	100	9	100	4	100	......	......	20	100
0.000501-0.00061	......	......	2	100	3	100	2	100	......	......	7	100
Solutions in serums.												
0.000221-0.00027	2	50	4	25	5	40	1	0	......	......	12	33
0.000271-0.00033	4	75	11	64	8	75	1	0	......	......	24	67
0.000331-0.00041	3	67	15	93	3	100	......	......	......	......	21	90
0.000411-0.0005	......	......	21	90	2	50	......	......	......	......	23	87
0.000501-0.00061	......	......	9	100	4	100	......	......	......	......	13	100
Cresol solutions.												
0.00018-0.00022	5	0	......	......	4	0	1	0	......	......	10	0
0.000221-0.00027	7	29	6	17	10	30	1	0	1	100	25	28
0.000271-0.00033	5	40	17	83	12	58	2	50	1	0	37	65
0.000331-0.00041	......	......	29	76	11	64	1	100	......	......	41	73
0.000411-0.0005	1	100	17	100	9	100	......	......	......	......	27	100
0.000501-0.00061	......	......	8	100	4	75	......	......	......	......	12	92

TABLE 10.—*Weight fatalities in mice inoculated with glycerine* (*206 mice*).

Dosage in grams per gram mouse.	12 to 15 gm.		15.01 to 18 gm.		18.01 to 21 gm.		21.01 to 24 gm.		24.01 to 27 gm.		Total.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
0.0075-0.009	11	9	12	17	12	17	10	10	2	0	47	13
0.0091-0.011	5	80	16	63	25	60	32	60	3	100	81	65
0.0111-0.0135	1	0	11	82	20	80	17	94	5	80	54	83
0.01351-0.0165	......	......	12	100	9	100	1	100	2	0	24	92

In order to get an idea of the susceptibility as influenced by weight, an index of susceptibility for each weight was calculated. This index for a given dose was taken as the geometric mean of the ratio of the mortality percentage for that weight and dose to the total mortality percentage for that dose, and the ratio of the total survival percentage for that dose to the survival percentage for that weight and dose.

M = percentage of mice dying on a given weight and dosage.
N = percentage of mice dying on all weights of same dosage.
U = percentage of mice surviving on all weights of same dosage.
V = percentage of mice surviving on given weight and dosage.

Then, the index of susceptibility for a given weight and dosage (as compared with the weight susceptibility of mice of all weights on the same dosage) is $\sqrt{\frac{M}{N} \times \frac{U}{V}} = s$.

To get the index of susceptibility of all the mice of a given weight each value "s" was multiplied by the lowest number of mice "n" used as a factor in obtaining the dose index. The sum of these products $(s \times n)$ divided by the sum of the n's gives the weighted index of susceptibility "S" for all the mice of a given weight. An index of 0.5 for mice of a given weight would therefore, by this measure, indicate that these have half the susceptibility of the mice of all weights, an index of 1 indicating that the susceptibility is the same and an index of 2.0 that mice of this weight are twice as susceptible as all the mice. From Table 11 it will be seen that there is little variation in susceptibility for animals of different weight.

TABLE 11.—*Susceptibility of mice of different weights to phenoloid preservatives and glycerine when administered in doses proportionate to body weight.*

Weight.	12 to 15 gm.	15.01 to 18 gm.	18.01 to 21 gm.	21.01 to 24 gm.	24.01 to 27 gm.
Index of susceptibility to phenol and cresols......	0.9	1.0	1.0	0.9	1.0
Index of susceptibility to glycerine..............	1.1	1.0	.9	1.1	.9

The customary method of calculating doses in grams per gram body weight would therefore appear reasonable for mice weighing between 12 and 27 grams.

SEX SUSCEPTIBILITY.

After the experiments had been started it was suspected that the uniformity of the results might be affected by the sex of the animals. Therefore a series of 32 mice, 16 males and 16 females, were inoculated with corresponding amounts of glycerine. No difference could be detected. However, in the remaining experiments the sex of all animals was recorded.

The mortality percentage of male and female mice on different doses were tabulated as shown in Tables 12 and 13.

TABLE 12.—*Sex susceptibility of mice inoculated with phenol and cresols.*

Dosage in grams per gram mouse.	Male.		Female.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
Aqueous phenol solutions.				
0.000221–0.00027	20	25	21	43
0.000271–0.00033	33	61	16	62
0.000331–0.00041	37	86	23	83
0.000411–0.00050	20	85	15	87
Aqueous trikresol solutions.				
0.00018–0.00022	6	0	13	0
0.000221–0.00027	21	57	17	35
0.000271–0.00033	32	81	14	86
0.000331–0.00041	32	97	16	100
0.00041–0.00050	7	100	1	100
Solutions in serums.				
0.000221–0.00027	2	50	10	30
0.000271–0.00033	13	85	11	45
0.000331–0.00041	5	100	16	68
0.000411–0.00050	10	100	13	77
0.000501–0.00061	4	100	9	100
Cresol solutions.				
0.00018–0.00022	2	0	8	0
0.000221–0.00027	15	13	10	50
0.000271–0.00033	23	70	16	62
0.000331–0.00041	21	86	19	58
0.000411–0.00050	13	100	14	100
0.000501–0.00061	6	100	6	83

TABLE 13.—*Sex susceptibility of mice inoculated with glycerine (102 mice).*

Dosage in grams per gram mouse.	Male.		Female.	
	Mice injected.	Per cent of deaths.	Mice injected.	Per cent of deaths.
0.0075 to 0.009	19	10	14	7
0.0091 to 0.011	14	57	21	62
0.0111 to 0.0135	12	83	12	83
0.01351 to 0.0165	10	100	10	100

From these data the index of susceptibility of male mice as compared with female was calculated for each dosage as in estimating relative weight susceptibility. The index of male susceptibility for all dosages was summarized, using the lower number of male or female survivals or deaths to weight the indices for each dosage.

The summarized index "S" for phenol and cresols gave a value of 1.2 indicating the males to be somewhat more susceptible than females. A similar computation of the index of male susceptibility for mice inoculated with glycerine gave a value 1.0 indicating little difference in sex susceptibility. However, only one-sixth as many mice were used on glycerine as on the phenoloids.

Other factors, such as age, diet, temperature, and method of caging, may have an appreciable influence on toxicity and lead to different results in different laboratories. Accurate differentiation by age is not practicable in routine work and no studies based on age were made; the mice used in these experiments were bred from the same stock and were about 5 weeks old. After inoculation they were kept in open-top glass battery jars and fed with moistened bread and oats.

It is believed that in comparing results under different conditions and with various poisons, as much respect should be paid to the broad range of toxicity as shown in charts 1 and 2 as to any other factor.

SUMMARY.

The following conclusions are drawn from the experiments detailed above:

1. (*a*) There is no definite minimum lethal dose of these disinfectants since some mice survive doses twice the size of those which kill a considerable proportion.

(*b*) Assuming the MLD to be the amount necessary to kill 80 per cent of the mice on a given dose, phenol has an MLD of 0.00037 gram per gram weight of mouse.

(*c*) Trikresol has the same MLD as found for phenol. This dose for phenol and trikresol is in substantial agreement with that found by Hale.

(*d*) The toxicity of trikresol and phenol is not lessened when these are diluted with normal horse serum.

(*e*) The toxicity of glycerine calculated on the basis of 80 per cent mortality is approximately 0.012 gram per gram weight of mouse.

(*f*) The toxicity of glycerine-phenol and glycerine-trikresol mixtures seems to be slightly higher than that of pure glycerine.

(*g*) In determining the toxicity coefficient of a coal-tar disinfectant it would seem necessary to use at least five mice on each dose; 20 per cent intervals between the doses represent the smallest spacing consistent with the accuracy of the method.

2. Mice inoculated with the coal-tar disinfectants died in two groups. The first period of death was 15 to 60 minutes and included one-sixth to one-third of the fatalities. The second period was between 1 hour 30 minutes and 4 hours, and included practically all of the remaining deaths. Mice inoculated with glycerine died between 30 and 90 minutes.

3. No difference in toxicity was shown in different dilutions of trikresol or glycerine. Phenol seems to be more toxic in the higher dilutions.

4. The susceptibility of white mice to soluble coal-tar disinfectants and to glycerine is not influenced by the weight of the animals if the doses are proportional to this weight and if the mice weigh between 12 and 27 grams.

5. Male mice are, roughly, 20 per cent more susceptible to the coal-tar disinfectants than are female.

OBSERVATIONS ON THE SIGNIFICANCE OF ANTISHEEP AMBOCEPTOR IN HUMAN SERUM, WITH REFERENCE TO COMPLEMENT FIXATION TESTS FOR SYPHILIS.[1]

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It is a common opinion among workers with the Wassermann reaction, perhaps especially among those who prefer to utilize human red cells rather than those of the sheep, that many of the alleged discrepancies in the results attained by the two methods may be explained on the following grounds:

That nearly all human sera contain some antisheep amboceptor, and that some human sera contain very considerable amounts.

That inasmuch as this is the case, preliminary attempts at the adjustment of the sheep cells hæmolytic system by titration, may fail to accurately balance the several factors of the reaction in the final tests. Thus it has been considered that sera containing relatively small amounts of syphilitic amboceptor may yield negative or doubtful reactions where positive results should have been obtained.

The fact is well established that many human sera contain antisheep amboceptor. Therefore the following tests were devised with the object of showing what effect upon the reactions given by positive syphilitic sera would be caused by artificially increasing the amount of antisheep amboceptor in these sera.

The experiments consisted essentially in placing diluted antisheep amboceptor (rabbit serum) in graded amounts in a series of test tubes. To these tubes constant amounts of positive syphilitic sera were added and complement fixation tests were performed. Complement and antigen in suitable amounts and dilutions were added. The tubes were subjected to the preliminary phase of the incubation for one hour at 37° C. Sensitized sheep cells were then added, the test incubated a second time for one hour, and placed in a 15° C. over night.

In performing the tests certain essential details of the method, following the technique elaborated by Stimson,[2] were employed.

The unit of amboceptor was taken as a constant. This was originally determined as follows: A test was set up in which graded amounts of complement serum were mixed with graded amounts of amboceptor serum in the presence of 1 c. c. sheep cell suspension. The curve of complete hæmolysis was plotted after one hour's incubation and the unit selected from the beginning of the relatively flat portion of the curve. The use of fresh amboceptor serum was avoided. Thus it was found that deterioration of the amboceptor serum was negligible.

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[1] Manuscript submitted for publication Jan. 3, 1917.

[2] Stimson, A. M.: 1915. Complement fixation in tuberculosis. Hygienic Laboratory Bull. 101, pp. 16-18.

Amboceptor and 5 per cent suspensions of sheep cells were considered as the standards for the adjustment of the hæmolytic system. Complement was that of guinea pig serum and the unit of complement was determined by titration against these standards just before the fixation tests were made. Two units of complement were used in the tests.

A 5 per cent sheep cell suspension was used. Amboceptor serum was added to this in such quantity that the amounts to be placed in each test tube (1 c. c.) should contain 1 unit of amboceptor. One portion of the suspension was used in determining the unit of complement while another portion of the same suspension was added to the fixation tests after the first phase of the incubation in 1 c. c. amounts.

An antigen composed of acetone insoluble lipoids prepared from beef heart was used after the customary titrations for antigenic and anticomplementary properties.

The positive syphilitic sera were inactivated just before use at a temperature of from 54° to 56° C. for one-half hour.

The total volume of the tests was 4 c. c.

Readings were made with the aid of a colorimeter, comparing the amounts of hæmolysis which had occurred in the various tubes with a completely hæmolized specimen. Results were recorded in percentages of fixation by assuming that in any given instance the percentage of fixation equals 100 minus the per cent of hæmolysis.

Throughout the experiments the direct measuring of minute amounts was avoided.

The following controls were set up for each test: Anticomplementary properties of the sera were controlled by adding twice the largest amount of serum used in the test to the hæmolytic system. If in any case this tube showed incomplete hæmolysis, the results were rejected for the entire test. Anticomplementary properties of antigens were controlled by adding double amounts to the hæmolytic system. Usual controls for the hæmolytic system and for friability of the cells were used.

Before reporting the results of the tests it is desired to give some account of the amount of natural human antisheep amboceptor in the sera utilized. At the same time with the fixation tests a series of tubes was set up containing the same cells as in the fixation experiments—of course, unsensitized. Two units of complement were added with varying amounts of the syphilitic sera and sufficient 0.9 per cent saline added to make the total bulk 4 c. c. The test was incubated one hour in 37° C. water bath.

Results given in percentages of hæmolysis are as follows:

TABLE I.—*Percentage of hæmolysis indicating presence of "natural" antisheep amboceptor in syphilitic serum.*

Serum No.	Amounts sera used in c. c.—			
	0.2	0.1	0.05	0.025
2086	....	100	90	80
2308	....	100	100	95
2375	95	85	60	30
2376	100	100	100	75
2377	85	75	50	20
2379	95	80	80	80
2380	100	95	70	40
2176	75	60	35	....
2875	90	90	85	50
2877	85	75	65	30
2878	90	80	65	30
3151	90	80	55	30

The results of the fixation experiments showing the influence of antisheep amboceptor as contained in sera on the Wassermann tests are reported in two tables. In the first, results are shown where the positive syphilitic serum was used in 0.1 or 0.2 c. c. amounts, as in routine tests; in the second much smaller amounts were used. In other words, these represented the antibody content of weak sera. Otherwise the work was done in exactly the same way.

Table II.—*Percentages of fixation obtained when the following amounts of antisheep amboceptor were added prior to the first phase of incubation and the usual amounts of syphilitic sera used.*

Serum No.	Amount (c. c.).	No amboceptor added.	Amount of amboceptor added.						
			1 unit.	2 units.	3 units.	4 units.	5 units.	7½ units.	10 units.
2086	0.2	100	100	100	100	100	100	100	100
2308	.2	100	100	100	100	100	100	100	100
2377	.1	100	100	100	100	100	100	90	90
2379	.1	100	100	100	100	100	100	100	100
2380	.1	100	95	90	85	80	75	65	55
2476	.1	100	100	95	90	85	85	75	70
2875	.1	100	100	100	100	100	85	80	80
2877	.1	100	100	95	95	95	90	85	80
2878	.1	85	80	60	60	40	30	20	10
3154	.1	100	100	100	100	100	100	100	100
3386[1]	.1	100					95		90
3386[1]	.2	100					95		90
3371[1]	.1	100					90		85
3371[1]	.2	100					95		90
3376[1]	1	100					85		80
3382[1]	.1	100					95		90
3382[1]	2	100					100		100

[1] Natural antisheep amboceptor removed by absorption by sheep cells before test.

Table III.—*Percentages of fixation obtained when reduced amounts of syphilitic sera were used.*

Specimen No.	Amount serum used (c. c.).	Per cent fixation obtained in ordinary Wassermann tests with this amount.	Per cent fixation obtained when 5 units amboceptor added preliminary to first phase of the incubation.	Per cent fixation when 10 units amboceptor added preliminary to first phase of incubation.
2086	0.006	100	80	65
	.005	100	80	70
	.004	100	70	40
	.003	95	20	15
2308	.035	85	40	20
	.025	70	35	0
2375	.06	100	55	15
	.05	100	45	30
	.03	.....	30	20
2376	.05	100	75	60
	.04	100	65	(1)
	.03	100	40	45
2377	.035	100	80	75
	.03	100	80	75
	.025	100	70	70
	.02	100	75	50
	.015	100	70	25
2379	.05	100	95	85
2380	.075	95	75	85
	.05	85	50	45
2476	.05	100	55	50
	.025	75	40	30
Syphilitic rabbit No. 3[2]	.022	100	75	50
	.02	95	60	40
	.018	85	55	45

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[1] Omitted.

[2] Rabbit tested for Wassermann reaction a few days subsequent to inoculation with spirochetes, reaction negative.

Attention is directed to the fact that the sera considered in Table I contained definite amounts of "natural" antisheep amboceptor. This was not removed before performing the fixation tests. Therefore the actual amounts of antisheep amboceptor present as shown in Tables II and III are greater than indicated.

It is conceivable that when 10 units or more antisheep amboceptor are present in the tests hæmolysis may be retarded and the appearance of fixation may result, i. e., the Neisser-Wechsburg phenomenon. It seemed possible that this might partly account for the lack of any very considerable increase in the hæmolysis with the presence of increasing amounts of amboceptor (see Tables II and III). This possibility was tested by (1) adding 1 unit of complement and 10 units of amboceptor to the usual amount of sheep cell suspension, (2) by performing fixation tests as described above using 10 units of antisheep amboceptor and a serum negative for syphilis. Hæmolysis was complete in both instances; therefore it seems unlikely that the large amounts of amboceptor present caused spurious fixation.

RESULTS OF FIXATION EXPERIMENTS.

Reference is made to Table II where positive syphilitic sera were used in 0.1 c. c. or 0.2 c. c. amounts as in ordinary Wassermann tests. In fact, such tests were actually performed as part of the experiments and appear in the first of the columns showing percentages of fixation. As the amounts of antisheep amboceptor in the human sera were artificially strengthened, the following results appeared:

I. With syphilitic sera of fairly high antibody content the presence of considerable amounts of antisheep amboceptor did not impair the amount of fixation. Sera Nos. 2086, 2308, 2379, 3154, 3382 (0.2 c. c.).

II. With syphilitic sera of moderate antibody content when used in 0.1 c. c. or 0.2 c. c. amounts, the presence of 10 units or more of antisheep amboceptor in these sera did not sufficiently impair the fixation so that a negative reaction resulted. Sera Nos. 2377, 2380, 2476, 2875, 2877, 2878, 3386 (0.1 c. c.), (0.2 c. c.), 3371 (0.1 c. c.) (0.2 c. c.), 3376, 3382 (0.1 c. c.). All these sera gave "strong positive," i. e., 70 to 100 per cent fixation, in the ordinary Wassermann tests. With 10 or more units of antisheep amboceptor added to them, 10 still gave "strong positive" reactions, while 2 were reduced to positive, i. e., 40 to 70 per cent fixation.

An examination of Table III reveals the following situation when antisheep amboceptor was artificially added to small amounts of positive syphilitic sera:

I. Using the 25 serum dilutions in ordinary Wassermann tests "strong positive" reactions resulted in each instance.

II. Adding 10 units of amboceptor to the diluted sera fixation was reduced from "strong positive" to "positive" 11 times; from strong positive to weak positive, i. e., from 20 to 40 per cent fixation, 5 times; and from strong positive to negative, i. e., less than a 20 per cent fixation, twice.

CONCLUSION.

While it can not be denied that natural antisheep amboceptor does reduce fixation when present in positive syphilitic sera, it would appear from the above experiments that the amount of antisheep amboceptor must be quite considerable and the amount of syphilitic amboceptor very small in order to produce a significant effect. It is therefore concluded that the presence of antisheep amboceptor in sera is not a valid objection to the use of a sheep cell hæmolytic system in performing routine complement fixation tests, provided the sera to be tested be used in amounts corresponding to not less than 0.1 c. c. for a test with a total volume of 4 c. c. or 5 c. c.

EXPLANATION OF CHARTS.

Percentage of fixation obtained by artificially increasing the amount of antisheep amboceptor in positively reacting syphilitic sera.

CHART 1:- SERUM 2086.

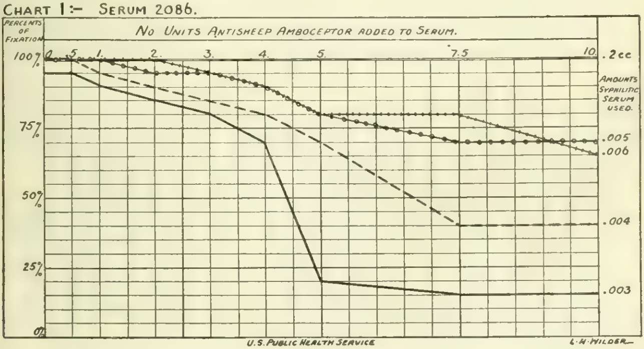

CHART 2:- SERUM 2308.

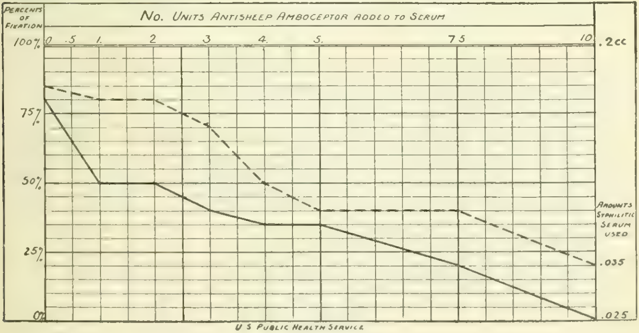

CHART 3:- SERUM NO. 2375.

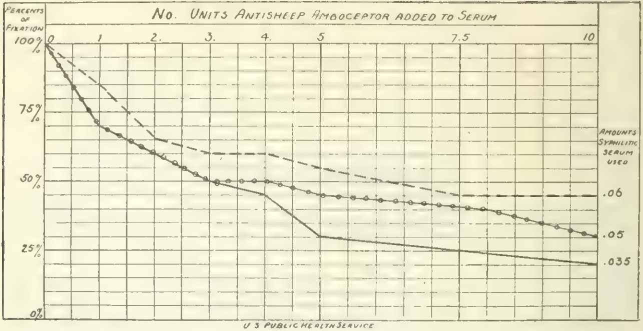

CHART 4:- SERUM NO. 2376.

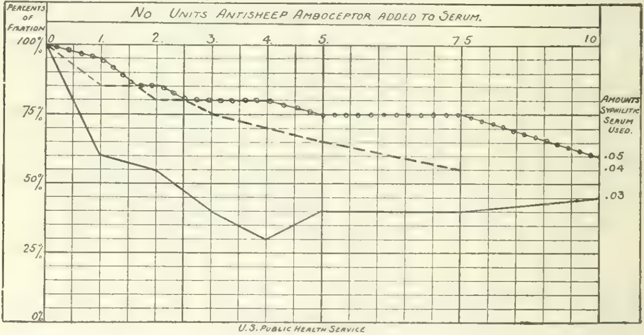

CHART 5:- SERUM NO. 2377.

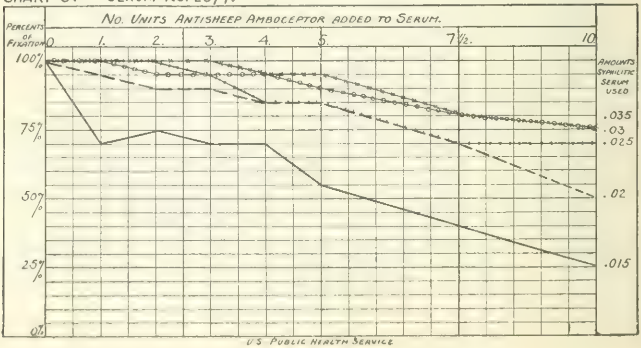

CHART 6:— SERUM No. 2380.

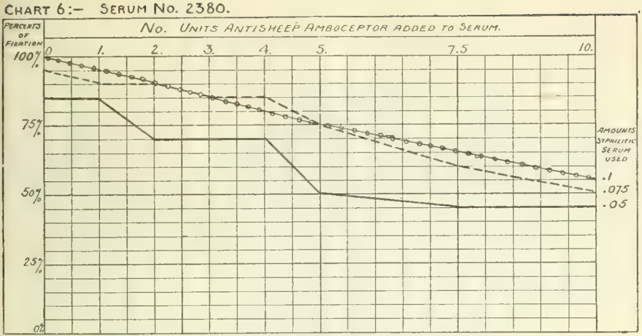

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CHART 7:— SERUM No. 24/0.

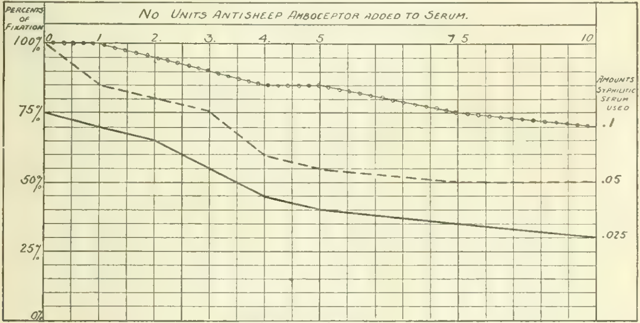

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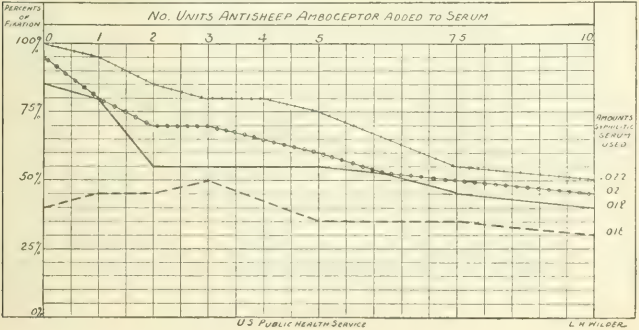
CHART 8:— SYPHILITIC RABBITS SERUM.



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HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

†No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

*No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis, Microphotography with simple apparatus, by **H. B.** Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

†No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

*No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

*No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

†No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

*No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

*No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lypolytic hydrolysis of ethereal salts. By J. H. Kastle.

*No. 27.—The limitations of fomaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

†No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

*No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

†No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

*No. 33.—Studies in experimental alcoholism. By Reid Hunt.

†No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

†No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

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TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY BULLETIN No. 111

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By N. E. WAYSON

II. EXPERIMENTAL POLIOMYELITIS

By J. P. LEAKE

III. ATTEMPTS TO INDUCE POLIOMYELITIS IN SMALL LABORATORY ANIMALS

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United States Public Health Service.

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I. THE PATHOLOGY AND PATHOGENESIS OF MYELITIS.

A DISCUSSION OF THE LITERATURE, WITH ITS BEARING ON EPIDEMIC POLIOMYELITIS.

By N. E. Wayson,

Assistant Surgeon, U. S. Public Health Service, Washington, D. C.

(From the Hygienic Laboratory.)

The conception of myelitis amongst the older writers, such as v. Leyden, was comprehensive, including the parenchymatous degenerations and system scleroses. Later observers limited the use of the term to an acute infectious process, more particularly of the blood vessels. Virchow, in 1897, adopted the older broad view of inflammation of the cord, based on the anatomical classification; while Hoche, in 1899, took exception to this, regarding the condition from the genetic and etiological viewpoint. Marinesco, in 1900, describes myelitis as a reaction of the tissues of the cord to an infection or toxin, present in the cord, in which reaction one or another tissue may predominate.

Dependent upon the anatomic-pathological conception, adopted by any given writer, acute myelitis was considered rare or common, according to whether it was primary, or secondary to an infection. Clinical observations had suggested its occurrence commonly as a secondary manifestation.

The adoption of the view, as stated by Pierre-Marie, that the genesis of the disease, rather than the anatomical changes, should be considered of prime importance, stimulated in the decade 1887–1897 much work on the experimental production of the various types of myelitis.

Inert and chemically active substances were injected into animals, but the influence of the bacteriological era brought the use of pathogenic organisms into greater prominence.

The more common pathogenic bacteria were introduced through the various routes of entrance to the cord; indirectly through the blood or lymph circulation, and directly by the intracerebral or intraspinal route.

Charrin used Pyocyaneus cultures injected intravenously.

Bourges injected streptococci from a case of erysipelas, both subcutaneously and intravenously.

Gilbert and Lion used the intravenous route, injecting colon bacilli.

Thoinot and Masselin used the intravenous route, injecting staphylococci and colon bacilli.

Widal and Besançon used the intravenous and subcutaneous routes.

Homén produced infiltrative myelitis by the injection of streptococci into the spinal canal, and also by the injection of the ischiatic nerve.

Ballet, reviewing the work done in experimental myelitis for the 10 years previous to 1897, including his own, makes these general statements on the histology:

> Nearly always the lesions are diffuse, with predominance in the enlargements and sometimes exclusive localization in the lumbar enlargement. The white substance is ordinarily but slightly involved. The alterations show a preference for the gray matter. The findings in a cord section of an acute ascending paralysis were: Congested vessels, with rupture of the walls, and hemorrhages; obliteration of the lumen and intense embryonic infiltration in the surroundings of the vessel. The ganglionic cells are vacuolated, the nucleus atrophied, with occasional lysis of the dendrites. Dilation of the arteries and hemorrhages are the rule; endocarditis and obliteration of the lumen and embryonic perivascular infiltration the exceptions.

He finds a wide variation in the lesions produced by organisms of the same type, and ascribes it to the virulence. He remarks that one determines medullary lesions much less readily with the pneumococcus or the typhoid bacillus than with the streptococcus and the colon bacillus. Cold, fatigue, and congenital susceptibility play a probably secondary rôle in these infections. He believes that the common organisms (staphylococcus, streptococcus, pneumococcus, and colon bacillus) become of increased pathogenicity.

Lamy devised a unique method for producing emboli in the cord by injecting into the abdominal aorta various foreign substances in such manner as to avoid emboli in the abdominal viscera: A laparotomy was performed and the abdominal aorta compressed immediately below the branching of the renal vessels and above that of the spermatic. This isolated portion of the aorta was injected through a catheter passed into it through the femoral artery.

Hoche enlarged upon Lamy's work, and Marinesco repeated Hoche's work, amplifying it by intraspinous and intraneural injections.

Hoche found that by the production of emboli in the vessels of the cord, with inert substances, such as lycopodium and starch granules, he could reproduce not only the clinical symptoms of myelitis but also the histology found in most acute cases. Further, by the injection of pathogenic organisms and chemical irritants, such as croton oil, he was able to produce conditions simulating in important details, both clinically and histologically, those of an acute anterior poliomyelitis and of an acute ascending myelitis.

Marinesco produced an infiltrative myelitis, predominating in the anterior horns, by the injection of streptococci into the blood vessels; and a peripheral myelitis, together with a meningitis, by injection of streptococci into the arachnoid spaces.

Vulpian, experimenting with emboli caused by injecting into the aorta starch granules and lycopodium, observed the predominant involvement of the gray matter. Hoche and Marinesco also noted this in their injections of the arterial stream.

The results obtained by most investigators using other routes were not predominantly lesions of the gray matter, and the changes produced were those of softening following emboli and thromboses rather than infiltration or proliferation around the vessels. A composite of their results would, however, reproduce the changes found in the various types of myelitis.

Ernst describes the histopathology of myelitis as follows:

> Like every inflammation, myelitis is associated with degenerative, circulatory (exudative and infiltrative), and proliferative disturbances, and, according to the presence of the one or the other, different forms may be distinguished. Moreover, the succession of these disturbances may vary, since it is evident (1) that an injury may affect vessels and tissues at the same time, therefore, may induce degenerative and circulatory changes, or (2) that the vessels suffer primarily and the vascular changes cause degeneration, that (3) finally the delicate and sensitive specific tissue elements undergo degeneration, the decomposition products of which act chemotactically upon the vessels and blood, and in this way cause exudation and infiltration. Which course is developed is not possible of determination in the individual case, still general pathology shows examples of all three ways.
>
> If the parenchymatous degeneration (the term degenerative myelitis is better than parenchymatous) predominates, then the cut surface is distended over the border, the axis cylinder and myelin sheath are swollen, vesicular spaces (areolae) and (Körnchenzellen) granular cells, with axis-cylinder fragments, appear in the tissue, the swollen glia appears as thick masses, even homogeneous, to which is added, as a result of further degeneration of the fatty detritus, myelin flakes, tigrolysis in the ganglia cells, disappearance of the processes, displacement toward the border, and degeneration of the nuclei, vacuolar degeneration and decomposition of the cells. The vascular changes (exudative myelitis) are characterized by a mottled reddening, small-cell infiltration with polynuclear leucocytes (in chronic inflammation with mononuclear round cells), and accumulation of emigrated cells in the perivascular and pericellular spaces; by transudation and oedematous swelling, by vascular dilation, hemorrhage up to the formation of infarcts (Myelitis hemorrhagica, apoplectica) cellular and hyalin thickening of the vascular wall, particularly the intima and adventitia. The more the infiltration is distinguished by cells, the sooner the myelitis is transformed into softening (with blood extravasation into red softening) and ragged areas appear filled with a pulpy, yellowish mass of myelin and axis-cylinder fragments.
>
> However, it is to be noted that softening may originate in other ways, for example, by colliquation necrosis with oedematous swelling, or by blood occlusion (ischemia), without our being able to determine in every case how it originated. Regeneration is associated with the destruction, and since the

specific nervous tissue remains inactive, it is left to the cleansing activity of the scavenger cells and to the constructive reparative growth of ameboid and fixed derivatives of the connective tissue and glia. In this way are formed connective-tissue cicatrices and neuroglial scleroses, which for a long time contain blood pigment, granular cells and amyloid bodies as the remains of the inflammatory process. The presence of bacteria is not the rule, so that the exciting cause of the inflammation must be sought for chiefly in their toxic products, since in experiments, not only with many well-known bacteria but also with toxins, it has been possible to induce myelitic changes.

Ernst classifies these changes in accordance with their localization, or better with their predominance, into transverse myelitis, meningomyelitis, ascending myelitis, poliomyelitis, etc., thus indicating that the histological changes are the same throughout the central nervous system, depending for their clinical or pathological entity upon the intensity or preponderance of the changes in the different structures forming the cord.

Acute poliomyelitis was considered by Charcot and Joffroy as a primary affection of the ganglion cells, followed by secondary changes in the blood vessels and stroma; whereas Roger and Damaschino considered the blood vessel changes as primary, the ganglion cells necessarily degenerating secondarily.

Careful and detailed examinations of the histopathology in acute anterior poliomyelitis, in acute and recovered cases, were made by Wickman and by Harbitz and Scheel.

Wickman, from a study of nine cases, including the acute and regenerative stages, concludes:

1. Poliomyelitis acuta is due to an infiltrative myelitis. This appears in scattered foci and is therefore to be regarded as a disseminated myelitis. The disseminated character appears especially distinctly in the medulla oblongata and in the brain, where, in my cases, changes were always shown when they could be examined.

2. In addition to the infiltrative conditions, one observes the effect of the macroscopic œdema, which may be increased up to the formation of locally circumscribed foci.

3. In their occurrence the interstitial changes correspond neither to the individual ganglion cells nor to the different ganglion-cell groups. This is shown most distinctly in the medulla oblongata, where the changes are regularly more pronounced without than within the nerve nucleus.

4. In the spinal cords changes are regularly found outside of the anterior cornua, and in fact as well in the other gray substance as in the white columns and in the pia.

5. The process undergoes certain regular fluctuations. First, it is most pronounced in the enlargements of the cord. Second, in the upper lumbar and still more in the lower dorsal cord, the changes show a condition varying from the ordinary. At these levels they are usually not as pronounced in the anterior cornua as in the Clark's columns, where they may reach or even exceed the intensity which they attain in the anterior cornua.

6. These fluctuations in the intensity coincide with the variations of the vascular content.

7. The process depends most intimately upon the vessels, and herewith appears to be associated the infiltration of all vessels, arteries as well as veins, and central as well as peripheral.

8. A very pronounced dependence of the changes upon the arteria centralis does not exist.

Wickman, in his later work, emphasizes neuronophagy as a preponderating lesion in epidemic poliomyelitis.

Harbitz and Scheel, after examinations of 17 cases, in which much work was done on the brain stem, cerebellum, cerebrum, and basal ganglia—some root ganglia were studied—describe the distribution of the infiltrative condition of the blood vessels over the base of the brain as similar to that of tubercular meningitis. There is involvement of the basal ganglia and of the cerebrum, especially along the Sylvian fissure.

They offer these latter changes as the probable explanation of those cases of acute bulbar palsy and polioencephalitis (superior and inferior, a descending process which never reaches the cord), which they with others are inclined to look upon as types of the clinical picture of acute anterior poliomyelitis.

They found also infiltration around cranial nerves, occasionally in one of them, explaining those cases of individual cranial nerve palsies. They found no distinctive changes in the spinal ganglia.

The disease they regard as a meningo-myelitis.

Fr. Schultze pointed out the correlations between meningitis and poliomyelitis, and drew the parallel of pleurisy-pneumonia.

Schaffer, describing the pathology of rabies, states:

> The site of predilection of hemorrhages is the posterior horn. The leucocytic infiltration is spread over the entire spinal marrow, but is marked with especial intensity only in certain segments.
>
> Blood vessel changes showed smaller endothelial cells, with the lumen of the vessel diminished. The blood vessels, especially the larger, were filled with a thread-like clot.
>
> The inflammation occurs in two forms, in some cases a colossal infiltration occurs, so that the nerve elements are scarcely visible because of the round cells; while in others, in which softening and deep structural changes occur, the infiltration is sparse and is limited to the adventitia of the congested vessels. The infiltration involves also the nerve roots. In the septa of the smaller nerve bundles there are numerous leucocytes. The blood vessels are congested and show an adventitial infiltration.
>
> Always that segment of the cord shows the greatest myelitic changes, which corresponds to the infected nerve.

Jochman, in his description of the pathology of epidemic meningitis, observes that the changes in the brain and cord, though appearing macroscopically to be limited to a hyperemia, are marked by numerous collections of lymphocytes in the perivascular spaces of the vessels, and by very small foci within the brain substance (encephalitic).

Barker, in studying specimens from an epidemic of meningitis, found changes in the ganglionic cells of the anterior horns and of Clark's columns, tigrolysis and vacuolization.

As is evidenced by the previous quotations and extracts, the histopathology of anterior poliomyelitis is not qualitatively specific, but is that of an acute disseminated infection of the brain and cord.

In myelitis (including poliomyelitis) the preponderance of the changes in the grey matter is to be expected in the event of dissemination by the blood or lymph circulation. The peculiar localization in the lumbar cord is not specific, since this is frequently the site of the most intense involvement in meningitis, epidemic and sporadic.

Edema and hemorrhage would be more prone to occur in the loose tissue of the gray matter than in the white matter, where the numerous septa, radial and cross, not only serve as supports to the vessel walls but tend to confine any hemorrhage.

Goldscheider and Flatau, in experimental studies of hemorrhage in the spinal cord, found the usual tracts of the hemorrhage to approximate a cylindrical shape, and, if starting in the anterior horns, to spread superiorly and inferiorly in the grey matter, extending into the contralateral anterior horn and the homolateral posterior horn, with but little tendency to involve the white matter. The course taken by the injection was that of least resistance, and was correspondingly influenced by the course of the nerve fibers, their crossings and their collection into bundles.

It is easily conceivable that edema would be influenced by the same factors. Moreover, injections into the dorsal segments extended farther longitudinally than in the lumbar and cervical segments; this would be expected by reason of the greater number of nerve fiber barriers in the enlargements, as contrasted with the dorsal cord. Edema could extend rapidly through this loose structure of the dorsal cord in an ascending palsy.

The peculiar distribution of the infiltration may be attributed largely to the blood-vessel distribution. Kadyi, in his studies on the anatomy of the circulation of the spinal cord, found a most extensive anastomosis of the vessels in the meninges of the cord, so that an embolus would produce relatively slight or no changes; but the distribution within the cord is entirely different, there being no anastomoses. Further, the distribution of the main vessels, the branching of the arteria centralis, is very irregular and differs in the various cord segments. In fact, if one accept as an hypothesis that only the arteria centralis is concerned in the distribution of the poliomyelitic virus within the cord, practically every localization could readily be explained by the vagaries of the branches of these vessels. Each turns into the right or left half of the cord from the anterior sulcus and divides into its main trunks, ascending or descending in the cord, explaining perhaps the monoplegias in the extremities. Many of the branches take, in the lumbar enlargement, a course in the same plane as that of their entrance; those bending at right angles ascend or descend relatively only a short distance, supplying an area of 2 to 4 mm.; hence numerous vessels are required to nourish each lumbar segment. In the dorsal region there are but few straight branches (those extending in the same plane of entrance), and the ascending and descending trunk branches are relatively long, distributed over 12 to 14 mm. of the cord. The increased number of vessels in the enlargement, as opposed to the dorsal segments, could explain the more frequent involvement of the enlargement. The branches have a most tortuous and irregular course, overlapping and paralleling each other in distribution. For example, one group of ganglionic cells may be nourished by several vessels and another by a single vessel; one ganglionic cell may even be nourished by more than one vessel. This overlapping and paralleling of vessels makes cross-sectional study of very little value, and is perhaps the explanation of the findings that an infiltrated vessel passes through or close to its group of normal ganglionic cells.

Kadyi found cross-sectional studies most misleading in his researches into the anatomy of these vessels.

The terminal branches of the ascending and descending branches in the dorsal cord sections have a parallel course along each side of, and a little posterior to, the central canal, resembling a bundle of sticks, and this peculiar distribution may account for the not uncommon involvement of Clark's columns.

Again, the branches of the arteria centralis occasionally extend into the posterior horn, and, contrary to their usual distribution in the gray matter, into white matter, which might account for foci in these locations.

The branches from the peripheral vessels, which usually follow the septa and nerve fibers and are limited in distribution to the white matter, extend occasionally into the gray matter.

Hence, depending upon the route of entry, the peculiar architecture, gross and minute, of the spinal cord might determine the predominant localizations; the histopathology remaining much the same, the greatest variation would be produced by the virulence of the infecting organism.

Through clinical observations on post-infection and specific myelitis, including poliomyelitis, the respiratory, alimentary, and genitourinary tracts, together with the skin and peripheral nerves, have severally been suggested as the atria of infection. Cases of myelitis have been observed as sequelæ, or in the course of the various infectious diseases affecting primarily these systems, whether the infection be general or local, and as the result of direct infection of the peripheral nerves through deep wounds. Thus we find myelitis following tonsillitis and influenza, pneumonia and tuberculosis, typhoid fever and dysentery, cystitis and syphilis, smallpox and erysipelas, rabies and tetanus.

Experimentation has resulted in myelitides from infection of the nasal mucous membrane, the mucous membrane of the intestines, the blood stream through the veins, and of the subcutaneous tissues. The most constant results, however, have been those of direct infection of the arterial blood stream, the perineural spaces, and the subarachnoid spaces. Injection through these routes has yielded histological pictures, strikingly similar in certain respects to those of acute epidemic anterior poliomyelitis.

The manner of conveyance of the infectious material to the cord has been considered, as hematogenous, lymphogenous, and as contiguous extension.

Lamy, Rothman, Hoche, and Marinesco have experimented with a direct conveyance through the arterial system, and they are proponents of the mechanical theory of the establishment of a focus of infection within the cord by reason of the histological changes produced by comparatively inert emboli, by chemically toxic emboli, as well as by pathogenic bacteria.

Wickman concludes from his study on the pathogenesis of poliomyelitis that processes which indicate an embolic origin of the disease are not observed.

The previously mentioned workers (Lamy, Rothman, Hoche, and Marinesco) are advocates of the embolic origin of poliomyelitis, making deductions from their findings in experimental myelitis. There are points for discussion in this theory. From the distribution of the vessels and the thickness of the cross sectional preparations studied by Wickman, it is easily conceivable that thrombosed vessels may have been overlooked. Further, Wickman in his protocols states:

> In some of the vessels of the anterior horn one sees finely granular masses, which lie now full in the lumen, now on the wall, and which now and then contain a few leucocytes and red cells.

Again:

> Some of the pial vessels contain a homogeneous mass in the periphery of which round, clear holes were often seen.

Hoche produced the lesions of acute myelitis, which simulated in some important features epidemic poliomyelitis, as is evidenced in the following protocols:

> Dog XXIV. Small female, half-badger. Lumbar arterial injection of 3 cc. of broth culture of *B. coli* (diluted with salt solution) with the addition of a small knifepointful of cornstarch. During the day following, flaccid paralysis of the hind legs.

The animal gives the impression of being very sick, lies around apathetic, snaps at every touch of the hand.

Died 51 hours after injection.

Necropsy.—Macroscopic appearance: In the lumbar cord, free hemorrhage, posteriorly in the arachnoid space. The lumbar cord upon cross section shows yellowish-red softening. Over the entire length of the dorsal cord the gray substance is stained red; the vicinity of the central canal in places highly red; in addition, punctiform hemorrhages scattered in the grey substance. These changes extend upward to the lowest segment of the cervical cord.

Microscopic appearance: The lumbar enlargement and below it: Cross section in places very indistinct; central canal no longer recognizable as such, necrotic places, extensive hemorrhagic infarctions; in less strongly affected places the white substance in the state of wide-meshed distension; the blood vessels in part greatly dilated; in part with walls infiltrated with small cells. Between individual root bundles, posteriorly as well as anteriorly, are observed circumscribed areas with pronounced cell increase in the pia.

The vascular dilations, with hemorrhagic infiltrated necroses in the tissue, extend upward to the boundary of the lumbar and dorsal portions of the cord; from hereon the histological picture is modified in the upward direction.

The white substance no longer shows any changes, or only those in the immediate vicinity of the gray substance; but the gray column is modified in the whole length of the dorsal portion up to the cervical portion.

In all sections were found foci of pronounced small-cell infiltration scattered in the tissue and small hemorrhagic extravasations; the small vessels are in part in the state of pronounced increase of the elements of cellular wall in part their sheaths are filled with small and large round cells; the central canal shows notable changes; the ependymal ring is torn in many places, the neighboring vicinity loosened and traversed with collections of round cells; here and there in the central canal detached ependymal flakes and red-blood corpuscles are found. At all levels colon bacilli are demonstrable; in the lumbar cord large embolic plugs are observed in part in the vessels; in part free in the tissue; above this they are found only in the tissue lying together in groups; also in close proximity of the central canal. I found no bacteria in the meningeal spaces.

Dog. XXXVIII. Small male pointer. Lumbar injection of 5 cc. of a broth culture of *Staphylococcus pyogenes aureus* with an admixture of a small knife-pointful of sterilized cornstarch.

Next day flaccid paralysis of posterior extremities; animal gave the impression of being very sick, scarcely moved, and refused nourishment.

Death on the fourth day.

Necropsy immediately after death.

Macroscopic appearance: No free hemorrhage externally. Lumbar cord for the most part very soft; punctate hemorrhages in both gray and white substances, as well as glassy, translucent reddish places; in the gray substance hemorrhagic points extend upward to the middle of the dorsal cord.

Microscopical appearance: Pia in many places, but only in the veinity of the blood vessels, infiltrated with small cells; nowhere a superficial infiltration; the vessels themselves show in these places increase of the cellular elements in the outer coats. The arteries in the interior of the cord show only slight dilatation; but frequently their walls and their immediate vicinity are lined thickly with small round or triangular cells, with only traces of protoplasmic fringe around the nucleus. In general few free hemorrhages are found without coincident cellular infiltration in the tissues.

The white substance shows in the lowest part of the dorsal cord, chiefly in the posterior columns, but in the lumbar cord arranged without system in the greatest variety of places, wide-meshed tissue with swollen and detached fibers, without active involvement of the supportive substance; the foci (to be described) in the gray substance frequently encroach upon the white substance. In the lowest part of the dorsal cord there is an elliptical area in the right posterior cornu, limited by a symmetrical fibrous glial border, without an essential increase of nuclei, which is entirely filled with cells approximately of the same size (i. e., quite small round and triangular cells) among which are only occasionally to be found large vesicular granular cells and very few red-blood corpuscles. No framework in the cavity is evident; the contents are quite sharply contrasted to the smoothly contoured environment.

With Weigert's bacterial stain it is shown that in additon to the cellular elements the cavity contains staphylococci in very large numbers, but in the wall of the cavity and in its vicinity not a single coccus is demonstrable. The cocci are evenly distributed in the cavity between the cellular elements.

Focal accumulations of the same type of cell are found in irregular shapes throughout the entire lumbar cord, but, as already mentioned, not predominating exclusively in the gray substance; nowhere else is the contrast to the surrounding so pronounced as in the above-described place, and even the type of cell described is not exclusively represented, since granular cells and red-blood corpuscles are found with them.

In the upper lumbar cord such a focus involves the central canal; here the one posterior wall of the same is not formed of ependymal cells, but of the small, round cells of the focus. Staphylococci are found here even in the most external layers of cells which form the canal wall, and since here and there, even in large numbers, freely detached cells are demonstrable in the central canal, it is to be certainly assumed that staphylococci also pass into the lymph stream in the central canal.

At all levels of the lumbar cord, particularly where the described focal accumulations of small cells are found, staphylococci are demonstrable in the tissue; moreover, some small vessels show large emboli of staphylococcal masses, from which radially in all directons a distribution of the microorganisms into the surrounding tissue is conceivable.

In the soft membranes (pia and archnoid), even in places with distinct tissue irritation, the demonstration of staphylococci was not possible for me.

Not only are the findings in the lumbar cord those of transverse myelitis, but the extension into the dorsal and cervical cord, together with the findings in these locations, are similar to those of ascending poliomyelitis.

Homen and Marinesco experimented with conveyance by the perineural sheaths, and by direct injection into the subarachnoid spaces. Both workers produced myelitis; using a streptococcus, they found but little involvement of the meninges, and this usually confined to the surroundings of the vessels of the pia. The type of myelitis produced was infiltrative, with marked proliferation in the perivascular spaces.

The similarity of the histopathology and localization in the lumbar cord, of rabies and epidemic poliomyelitis, has suggested the probability of conveyance by the same routes, the perineural sheaths; Di Vesta and Zagari, Bardach, and Cantani have experimentally shown transmission of rabies virus by this route, and that the segment of the cord corresponding to the nerve injected was primarily and most intensely involved.

Wickman explains the conveyance of the virus of acute epidemic poliomyelitis to the cord through a lymphogenous route, either from the nasopharynx or the intestine.

Romer considers the absence of the virus in the blood stream, during the incubation of the disease, a point against hematogenous spread; its presence in lymph glands near a subcutaneous site of inoculation an argument in favor of lymphogenous carriage. Moreover, the portion of the cord corresponding to the nerve injected experimentally is first infected.

His reasoning is based on analogies in the manner of conveyance of rabies virus, and the meningococcus of epidemic meningitis, to the cord. These diseases each resemble in certain phases acute epidemic poliomyelitis.

Jochman is not sure of the lymphogenous spread of epidemic meningitis, because of the histological findings in the heart muscle and endocardium, together with the presence of the organism in the blood stream.

The constancy of the experimental findings in rabies, of involvement of the cord segment corresponding to the nerve infected, is not the picture of experimental poliomyelitis, as is evidenced by the preponderant localization in the lumbar cord in intracerebral injections.

Wickman offers as probable explanation of the preponderating involvement of the lumbar segment in infection in nature the spread through the intestinal mucosa to the sympathetic nerves, hence to the central nervous system radicals. However, both Wickman and Harbitz and Scheel find but slight or no involvement of the spinal ganglia infected by natural routes; nor is there the frequency of greatest involvement of the posterior horns, as would be expected; the spinal ganglia are involved in rabies. Wickman offers in opposition to the mechanical hematogenous theory the experience of Nonne, who found a predominance of localizations in the white matter in emboli from endocarditis, the lesions resembling those of a transverse myelitis; also Heller, Mager, and v. Schrötter, in their studies of gaseous emboli in caisson disease, found a predominance in the white matter. Wickman also points out the frequency of cerebral emboli as opposed to cord emboli, and, in the ascending type of the disease, the necessity of the progressive involvement by segments of the central arteries. Further, he fails to find any thrombi. Nor did he find capillary involvement; he did find the lesions along the veins. Römer emphasizes the point of Wickman's findings along the veins and the absence of capillary involvement.

Hoche found ascending changes, as cited in the above protocol, without progressive emboli. Goldscheider and Flatau found an extension of hemorrhages for 25–60 mm. longitudinally without progressive hemorrhages, and edema is always present in these acute circulatory changes.

With but few exceptions, the workers attempting to reproduce a proliferative meningomyelitis preponderating in the surroundings of the vessels over the base of the brain, anterior surface of the cord, and in the anterior horns of the gray matter have been unsuccessful except through perineural or intra-arachnoidal injection. The successful intraperitoneal and nasopharyngeal injections may be explained by a hematogenous or lymphogenous conveyance.

Wickman concedes the probability of either a hematogenous or lymphogenous conveyance, or both, but believes the lymphogenous more probable for the reasons stated. Harbitz and Scheel consider the lymphogenous route insufficient to explain the findings in the disease.

The spread within the cord has been explained by an extension along the central canal and along the perivascular lymph spaces, rather than by the outside route through the arachnoid spaces.

Harbitz and Scheel, accepting Schultze's view, are of the opinion that the virus settles in the meninges and makes its way into the cord along the vessels, a finding in correspondence with other meningitides.

The spinal fluid in poliomyelitis is relatively noninfectious, but recent workers claim to have found a specific streptococcus in the fluid. Since Homen's work it has been recognized that organisms disappear rapidly, not only from the spinal fluid but from the central nerve axis, explaining perhaps many negative findings.

Babes early called attention to the presence of bacteria in the central canal in conditions other than cord affections. Later observers of organisms in the canal, as Morel, Rispal, Homen, and Hoche were inclined to consider this route as a channel in the development of ascending paralyses (Landry type).

It was recognized that this channel was closed in adults, but attention was directed to the preponderance of poliomyelitis in children under 12 years of age, after which period the central canal is likely to be obliterated.

Wickman recorded a patent central canal in some of his adult cases of ascending paralysis.

Goldscheider and Flatau showed in their injection studies of hematomyelia that the central canal in adults was not a highway for the spread of hemorrhage.

Wickman, from his studies on poliomyelitis, concluded that the spread within the cord was by the perivascular lymph spaces. Kadyi, however, doubts the presence of any perivascular lymph spaces within the cord and attributes Hiss's findings of these spaces to artefacts.

The contributary etiological factors in myelitis are probably those which will produce a condition of lowered resistance in the cord, and include cold, trauma, fatigue, and congenital susceptibility.

The specific factors are pathogenic organisms and their toxic products. Babes observed various pathogenic bacteria—staphylococci, typhoid bacilli, and colon bacilli—within the central nervous axis, without histological changes, and believes the importance of their presence greatly overestimated. However, numerous workers have produced myelitis by the injection of various pathogenic organisms and their toxins, including the streptococcus, staphylococcus, typhoid bacillus, and colon bacillus, and some have used toxic substances, such as cancer juice. The streptococcus has been prominent in the writings of several authors as the producer of proliferative myelitis. Babes finds streptococci producing simple necrobiosis, perivascular infiltrations, or the chronic lesions of vascular and neuroglial scleroses with atrophy. Likewise, he finds streptococci in the cord in epidemics of hemorrhagic infection with acute paralysis among horses and in diseases of young dogs with lesions of anterior poliomyelitis. The streptococci are sometimes associated with other pathogenic organisms.

In epidemic poliomyelitis, diplococci have been found in the spinal fluid.

Geirsvold and Harbitz and Scheel had some striking findings of cocci during epidemics. Neither of these latter authors is convinced of the specificity of his findings.

However, constant results in the experimental reproduction of the disease by cultures have not been attained. Noguchi and Flexner isolated a minute filterable organism which arranged itself as diplococci and streptococci, and were able to reproduce the disease after several culture generations. Workers outside of their laboratories have thus far failed to confirm their work, with the probable exception of Rosenow. Rosenow believes he has isolated the organism found by Noguchi, and grown it into a polymorphic streptococcus, which produces the disease when injected into the venous circulation by reason of its selective or elective localization.

Wickman tried to adapt a streptococcus recovered from a tendovaginitis to a selective growth in the cord by injection into the spinal canal in the lumbar region and recovery from the cervical region, but in spite of 90 or more direct transfers from animal to animal was unsuccessful.

DISCUSSION.

Studies on the pathology and pathogenesis of myelitis show that the various hypotheses as to the genesis of its different forms have strongly supportive evidence, but no theory carries an overwhelming amount of this evidence. There seems to be, from the anatomic-pathological viewpoint, no reason for great dispute, since the various histological changes are seen in the different types of the condition, apparently dependent upon the rapidity of the process, in turn dependent upon the virulence of the infectious material for these structures. The localization in the cord seems dependent upon the mode of entrance and probably upon a specific factor (trauma in transverse myelitis). Extension through one or another system of the cord lends itself readily to the explanation afforded by anatomical structure.

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II. EXPERIMENTAL POLIOMYELITIS.

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EXPERIMENTS WITH VIRUS OF HUMAN ORIGIN OBTAINED DURING EPIDEMICS.

The sources of this material were two epidemics, one prevailing in the summer of 1916 (the material coming from New York City and vicinity), and the other in the winter of 1916–17 in West Virginia. The material consisted of portions of human spinal cord, removed at necropsy from definite cases of poliomyelitis, and preserved in glycerin.

The glycerinated virus was ground by hand in small mortars, with the addition of physiological saline solution to make an approximately 5 per cent emulsion. This was strained through gauze, and injected into the monkeys, 0.5 cc. into the left cerebral hemisphere, and 2 cc. intraperitoneally.

1. The results of the primary inoculations are shown in Table I. All of the seven viruses from New York City produced poliomyelitis in monkeys, three of which survived the acute attack. There were no failures, in spite of the fact that but a single monkey was used for each virus. Of the four New Jersey viruses, only one produced a typical attack, and from this the monkey recovered. Another produced dubious symptoms, and the remaining two were thrown out of consideration by the premature death of the animals, one from brain abscess and the other from an undetermined cause. Of the West Virginia viruses, two produced typical poliomyelitis with recovery, and the third no symptoms. The numbers of viruses composing these three groups are too small to warrant trustworthy conclusions.

TABLE I.—*Monkeys inoculated with human spinal cord.*

Human case.	Place of necropsy	Date of necropsy	Cord preserved in—	Stored before inoculation.	No. of monkey.	Incubation period.	Result.	Histological pathology.
		1916.		*Days.*		*Days.*		
A. V.	College Point, N. Y.	July 19	Pure glycerine	14	85	10	Typical symptoms; almost total paralysis; killed third day.	Classical poliomyelitis.
S. G.	New York City	July 20	do.	13	16	8	Paralysis nearly complete; killed third day.	Typical poliomyelitis.
W. McF.	New York quarantine.	July 21	Pure glycerine. Transferred July 25 to 50 per cent glycerine.	12	86	11	Complete paralysis hind limbs, partial front limbs. Survived.	
F. W.	do.	July 22	do.	11	131	8	All limbs paralyzed but left forefoot. Intercostals paralyzed. Killed on second day.	Positive poliomyelitis; used as stock strain.
S. E.	do.	July 23	do.	10	116	9	Paralysis right side complete, left partial, killed sixteenth day.	Some infiltration. Marked degeneration of ganglion cells and neurophagocytosis.
P. C.	do.	do.	do.	10	133	6	Paralysis hind limbs; partial in forearms, killed second day.	Typical poliomyelitis.
C. S.	do.	July 24	do.	9	121	6	Paralysis right forefoot, killed fortieth day.	Positive poliomyelitis.
1487	Newark, N. J.	July 29	50 per cent glycerine	9	78	5	Hind legs nearly symmetrically paralyzed. Survived.	
1488	Englewood, N. J.	July 30	do.	8	135		Second day trembling, fourth day dead.	Negative.
1494	Newark, N. J.	Aug. 3	do.	4	40	9	Questionable weakness; died seventieth day.	Do.
1498	do.	Aug. 4	do.	3	200		Died third day.	Brain abscess.
		1917.						
L. C. P.	Elkins, W. Va.	Jan. 7	do.	2	265	9	Paresis both hind legs. Recovery practically complete.	
W. E.	do.	Jan. 8	do.	1	266	9	Paresis both hind legs. Recovery complete.	
H. E. A.	do.	Jan. 12	do.	4	268		No symptoms.	

2. One of the New York viruses selected as a stock virus (F. W.) has been propagated through eleven "generations," in monkeys. The data of these passages are given in Table II. No intracerebral inoculation with this untreated strain has failed in our hands to produce poliomyelitis in monkeys.

TABLE II.—*Passage of N. Y. virus (F. W.) from monkey to monkey.*

Monkey No.	Generation in monkey.	Material inoculated.	Incubation period.	Duration of symptoms.		Result.
			Days.	*Days.*	*Hrs.*	
131	First....	5 per cent emulsion of human cord (F. W.) glycerinated 11 days.	8	1	12	Practically complete paralysis. Killed.
211	Second..	2½ per cent emulsion of fresh cord of monkey 131 kept at 37° C. 1 hour and at 15° C. for 19 hours.	6	1	6	Do.
219	Third...	5 per cent emulsion of cord of monkey 211 glycerinated 44 days.	4		18	Fatal.
220	...do....	.....do.	5		22	Do.
221	...do....	.....do.	5	3		Nearly complete paralysis. Killed.
222	...do....	.....do.	5½		18	Fatal.
217	Fourth..	5 per cent emulsion of cord of monkey 221 glycerinated 32 days.	4	2		Nearly complete paralysis. Killed.
253	...do....	.....do.	7	3		Partial paralysis. Killed.
263	Fifth....	From monkey 253, glycerinated 42 days.	4	2		Practically complete paralysis. Killed.
264	...do....	.....do.	6	2		Partial paralysis. Killed.
267	Sixth...	From monkey 264, stored two days at 15° C.	4	1	12	Practically complete paralysis. Killed.
269	Seventh.	Cord and brain from monkey 267 emulsified fresh and stored 18 hours at 15°.	4	2		Do.
275	Eighth..	Brain of monkey 269, fresh..........	8	4		Partial paralysis. Died.
279	Ninth...	Brain of monkey 275, emulsified fresh and stored 20 hours at 5° C.	5	2		Partial paralysis. Killed.
255	...do....	.....do.	5	2		Do.
256	...do....	.....do.	5	2		Nearly complete paralysis. Killed.
282	Tenth...	Brain and cord from monkey 279, glycerinated 13 days.	4	1		Do.
[1] 254	Eleventh	Brain and cord of monkey 282, fresh..	5	2		Practically complete paralysis. Killed.
283	...do....	.....do.	4	2		Do.
284	...do....	Brain and cord of monkey 282 glycerinated 4 days.	5	2		Nearly complete paralysis. Killed.

[1] This monkey had, 84 days previously, received an intraspinal injection of fresh virus from monkey 279 without result.

The incubation period in monkeys inoculated directly with human virus averaged eight days, in passage monkeys five days. The shortest incubation period was four days, and the longest eleven days.

The distribution of paralysis was in the direction but not of the degree determined by Römer,[1] and Leiner and von Wiesner,[2] who found a much greater predominance of paralysis on the side opposite to the hemisphere inoculated. In 11 of our cases, where the paralysis was obviously asymmetrical in the beginning, the palsy was in fact more common on the side inoculated (6:5); but in 10 cases, where the paralysis was asymmetrical at its height, the inoculated side was less often the more severely involved (3:7). The accompanying intraperitoneal injection, usual in our series but not in those of Römer and of Leiner and von Wiesner, may have influenced these results. This difference may indicate that in some instances the virus from the abdominal cavity becomes effective as promptly as does that from the brain.

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[1] Römer, Paul H.: Epidemic Infantile Paralysis (Heine-Medin disease). Translation by H. R. Prentice, New York, Wm. Wood & Co., p. 99.

[2] Leiner, K., and von Wiesner, R. R.: 1910: Wien. klin. Wehnschr., vol. 23, no. 9, p. 323 (March 3).

3. Attempts to produce poliomyelitis in monkeys by injection of filtered nasal washings from possible human carriers were made in a few instances with negative results. (Table III.)

TABLE III.—*Inoculation with human nasal washings, filtered.*

Animal.	Human source.	Injection.	Result.
Monkey..	F. W., poliomyelitis 6 years previous...	80 cc. intraperitoneal, 2 cc. intraneural.	Remained well.
Rabbit...	.....do..................................	50 cc. intraperitoneal..............	Do.
Monkey..	M. W., mother of F. W., and in contact with B. H. prior to onset.	70 cc. intraperitoneal, 2 cc. intraneural.	Do.
Rabbit...	.....do..................................	50 cc. intraperitoneal.....	Do.
Monkey..	B. H., poliomyelitis 8 days previous....	60 cc. intraperitoneal, 2 cc. intraneural.	Do.

4. Attempts to produce poliomyelitis by transferring nasal secretions from sick to well monkeys were without result. Healthy monkeys placed in contact with those inoculated remained well. (Table IV.)

TABLE IV.—*Attempts at nasal infection.*

Monkey No.	Material (obtained just before killing the monkeys indicated, except that from No. 284).	Inoculation.	Result.
201...	Dry nasal secretion from poliomyelitis monkey 133. (See Table I.)	By platinum loop to posterior nares.	Remained well.
202	Placed in contact with monkey 201 ..	....	Do.
203	Dry nasal secretion from poliomyelitis monkey 133. (See Table I.)	By platinum loop to posterior nares.	Do.
204 .	Placed in contact with monkey 203 ..	....	Do
205	Dry nasal secretion from poliomyelitis monkey 133. (See Table I.)	By platinum loop to posterior nares.	Do.
206	Placed in contact with monkey 205 ...	....	Do.
207	Nasal secretion from poliomyelitis monkey 131. (See Table I.)	Cotton pledget soaked in secretion placed in right nostril.	Do.
208	.....do	.....do..............................	Do.
124	Profuse nasal secretion (after 0.1 gm. pilocarpine intravenously) from poliomyelitis monkey 16. (See Table I.)	Cotton pledgets soaked in secretion placed in left posterior nares.	Do.
215	.....do..................................	.....do..............................	Do.
216	Profuse nasal secretion (after 0.2 gm. pilocarpine intravenously) from poliomyelitis monkey 85. (See Table I.)	.....do..............................	Do.
217 [1]...	Profuse nasal secretion (after 0.05 gm. pilocarpine subcutaneously) from poliomyelitis monkey 211. (See Table II.)	Cotton pledget soaked in secretion placed in left nostril.	Do.
285 ...	Fresh cord of poliomyelitis monkey 284 (see Table II) ground without adding salt solution.	Swabbed gently on nasal mucosa..	Do.
286	.....do..................................	..do.................................	Do.

[1] This monkey proved susceptible to intracerebral inoculation 84 days later. (See Table II.)

5. Feeding poliomyelitis virus to monkeys failed to produce the disease. (Table V.) However, on subsequent intracranial inoculation one of the two monkeys so fed failed to come down with the disease at first and succumbed to the second inoculation after a slightly prolonged incubation period. The question as to whether the animal had been rendered partially immune by the feedings remains open.

TABLE V.—*Administration of virus with food.*

Monkey No.	Material.	Administration.	Result.	Intracerebral inoculation.	Result.
258.......	Ground brain of monkey 253 (see Table II) in banana.	First day, 15 gm.; second day, 4 gm.; third day, 10 gm.; fourth day, 15 gm. Morphine sulphate, 0.0006 gm., subcutaneously.	Remained well	104 days later..	Paralysis, 8 days' incubation.
259 [1]......	.....do............	First day refused to eat; second day, 3 gm.; third day, 10 gm.; fourth day, 15 gm.; morphine sulphate, 0.0006 gm., subcutaneously.	.....do........	104 days later. Second inoculation 152 days after administration of food.	Remained well. Fatal paralysis, 10 days' incubation.

[1] Large cynomolgus.

6. Attempts to produce the disease by combined intracerebral and intraperitoneal injections of filtered virus were successful in two out of three instances. The successful results were obtained with virus from a monkey, filtered in the one case through a Berkefeld N and in the other through a Chamberland B filter. Further particulars are given in Table VI.

TABLE VI.—*Filtration of the virus.*

Monkey No.	Material.	Filter.	Injection.	Bacteriological control of filtrate.	Result in monkeys.
287.....	5 cm. fresh cord of monkey 284 (see Table II) ground and diluted to 120 cc. with saline solution, mixed with living *B. prodigiosus* to give light pink color.	Berkefeld N.	1 cc. intracerebral; 6 cc. intraperitoneal.	Agar slant, aerobic and anaerobic broth gave no growth.	Paralyzed after 9 days. Survived.
292.....	.....do............	Berkefeld N (18 hours at 25° C.).	2 cc. intracerebral; 6 cc. intraperitoneal.	5 cc. in aerobic and anaerobic broth tubes, also long ascites-tissue-broth tubes gave no growth.	Remained well.
288.....	3 cm fresh cord of monkey 284 (see Table II) ground and diluted with saline to 100 cc.	S. & S. 588 and Chamberland B.	2½ cc. intracerebral; 25 cc. intraperitoneal.	2 cc. in aerobic and anaerobic broth tubes and 10 cc. in long ascites-tissue-broth oil covered tubes gave no growth as compared with uninoculated controls.	Paralyzed after 8 days; killed 4 days after. Typical histology. Cord caused poliomyelitis in 3 other monkeys after intracerebral inoculation.

7. An attempt to determine the immunity of a possible carrier by a serum neutralization test is shown in Table VII. The uncertainty of the test is evident in that 0.5 cc. of the possibly immune serum apparently neutralized 0.5 cc. of virus but failed to neutralize 0.1 cc. of the same virus. Of the control sera used that of J. P. L., which apparently neutralized the virus, was from a person who had repeatedly been in contact with acute cases of poliomyelitis within the previous month and also some years previously, but without showing any symptoms of infection. The other two sera used as controls, A. M. S. and N. E. W., failed to neutralize the virus, and were from persons who had not been in close contact with acute human poliomyelitis. Included in Table VII also are experiments showing failure to neutralize the virus by using either normal monkey serum or that of a monkey which was suffering from typical poliomyelitis of one month's duration.

TABLE VII.—*Neutralization tests* (*intracerebral inoculation*).

Monkey No.	Serum.	Virus.	Exposure.	Result.
209	0.5 cc. from B. (suspected carrier).	0.5 cc. fresh 5 per cent cord emulsion, from monkey 131. (See Table I.)	1 hour at 37°, 19 hours at 15° C.	Remained well except for slight questionable weakness 10 days later. No immunity to intracerebral inoculation (4 days incubation) 275 days later.
210	do	0.1 cc. fresh 5 per cent cord emulsion from monkey 131. (See Table I.)	do	Paralysis after 9 days, typical histology.
211	0.5 cc. 0.85 per cent NaCl solution (control).	0.5 cc. above virus	do	Paralysis after 6 days, typical histology.
212	0.5 cc. from A. M. S. (normal adult).	do	do	Paralysis after 9 days, typical histology.
213	0.5 cc. from J. P. L. (normal adult, see p. 28).	do	do	Remained well.
214	0.5 cc. from N. E. W. (normal adult).	do	do	Paralysis after 5 days. Survived.
223	0.5 cc. from monkey 121 (see Table I) 30 days after attack of poliomyelitis.	do	do	Paralysis after 8 days, typical histology.
224	0.5 cc. from fresh monkey	do	do	Do.

8. Attempts to immunize monkeys with the serum of a horse, which had received injections of poliomyelitis virus, were unsuccessful. (Table VIII.) This serum was furnished by Dr. Connelly, of Newark, N. J.

TABLE VIII.—*Attempted immunization with serum of treated horse.*

Monkey No.	Serum injections, intravenous.	Time of virus inoculation.	Virus.	Result.
253	First day, 5 cc. a. m., 5 cc. p. m; second day, 10 cc. a. m., 5 cc. p. m.; fourth day, 10 cc.; fifth day, 10 cc.	At fourth serum injection.	0.5 cc. from monkey 221. (See Table II.)	Paralysis after 7 days. Typical histology.
257	First day, 10 cc.; second day, 10 cc., third day, 10 cc.; fifth day, 10 cc.; seventh day, 10 cc.; ninth day, 10 cc.	24 hours after last serum injection.	0.5 cc. intracerebrally and 2 cc. intraperitoneally from monkey 133. (See Table I.)	Convulsions and ataxia on second day; died in 18 hours. Meningeal congestion and pulmonary infarct. No poliomyelitic changes in cord.
289	First day, 10 cc.; second day, 10 cc.; third day, 10 cc.; sixth day, 10 cc.; eighth day, 10 cc.; twelfth day, 10 cc., thirteenth day, 10 cc. a. m., 10 cc. p. m.	Half hour after last serum injection.	1 cc. intracerebrally and 2 cc. intraperitoneally from monkey 288. (See Table VI.)	Paralysis after 8 days. Typical histology.

9. The identity of the disease causing the summer and the winter epidemic was practically demonstrated by the immunity tests shown in Table IX. Monkeys surviving successful infections with the winter disease (West Virginia), were immune to the virus of the summer disease (New York).

TABLE IX.—*Immunity test.*

Monkey No	First treatment.	Result.	Second inoculation.	Result of second inoculation.
214	New York virus (summer epidemic) intracerebrally from first generation in monkeys. (Control).	Severe paralysis hind legs.	New York virus intracerebrally (twelfth generation in monkeys)	No symptoms from second inoculation.
265	West Virginia virus (winter epidemic) intracerebrally; direct inoculation from Elkins A necropsy.	Paresis hind legs with nearly complete recovery.	do	Do.
266	West Virginia virus (winter epidemic) intracerebrally; direct inoculation from Elkins B necropsy.	Paresis hind legs with practically complete recovery.	do	Do.
268	West Virginia virus (winter epidemic) intracerebrally; direct inoculation from Elkins C necropsy.	Remained well	do	Marked typical progressive paralysis after 11 days' incubation.
258	Fed on virus	do	do	Marked typical progressive paralysis after 8 days' incubation
259	do	do	do	Remained well.
289	Intravenous injection of serum from horse treated with poliomyelitis virus.	No symptoms.	do	Typical fatal poliomyelitis after 8 days' incubation.

EXPERIMENTS WITH MATERIAL FROM DOMESTIC ANIMALS ASSOCIATED WITH CASES OF HUMAN POLIOMYELITIS.

10. All of these animals (Table X), except the cat, had manifested symptoms of a paralytic nature; and all came from epidemic foci of poliomyelitis. In most instances, more or less intimate association

with human cases was traced. Inoculations were made into healthy animals of the same species, and in some cases into monkeys. No evidence was elicited to show that any of the animals had suffered from a disease corresponding to human poliomyelitis. In three ininstances, the material used showed histologically an involvement of the spinal cord or its membranes, but the condition was not transmitted by inoculation. In one instance, a dog, receiving an injection of spinal cord emulsion, developed paralysis associated with a hemorrhagic condition of the cord; but this was not transmissible to puppies. Further details are shown in Table X.

TABLE X.—*Material from pet animals associated with poliomyelitis.*

Animal.	History.	Material.	Histology.	Inoculated into.	Result.
Bull dog.	Two human cases in same house in New York City. The dog paralyzed in hind legs about the same time; necropsy 6 days later.	Spinal cord....	Very slight perivascular round cell infiltration, not as in poliomyelitis. (Kidney from this dog shows parenchymatous and fatty degeneration.)	3 puppies, intracerebrally.	Remained well.
French poodle.	Cases in neighborhood in New York City. Paralyzed in all legs, clinically distemper; necropsy 3 days later.	.....do........	Meningo-myelitis. Polynuclear and round cell infiltration of pia-arachoid extending into cord with vessels; marked in anterior commissure, in white matter, and about vessels in posterior part of cord; round cell infiltration in posterior fissure. No very marked changes in anterior horns.	Dog, intracerebrally.	Do.
Female bull dog.	Cases in neighborhood in New York City. Paresis hind legs with convulsions; necropsy next day.	...do.......	Normal..............	..do......	38 days later became paralyzed in hind quarters. At necropsy nothing abnormal was found except extensive hemorrhages (histologically) in the gray matter of the cervical and lumbar cord; subinoculations into three puppies were without result.
Fox terrier.	Human case in same household (College Point, N. Y.) 2 days after this dog was sick with vomiting and diarrhea. Apparently well when killed for necropsy 11 days after.	Spinal cord and nasal mucosa.	....	Fox terrier, intracerebrally.	Remained well for 32 days, when it was killed by the other dogs.
Cat......	Same household as above.	Salivary glands and nasal mucosa.	....	Cat, intracerebrally.	Remained well.

TABLE X.—*Material from pet animals associated with poliomyelitis*—Continued

Animal.	History.	Material.	Histology	Inoculated into.	Result.
Bull dog, 10 months old.	Two human cases in same household (New Haven) 10 days after this dog had an attack (lasting 12 days) of weakness in hind legs and loss of bark. Apparently well when killed for necropsy 38 days later, except for slight nasal discharge. Necropsy negative.	Nasal mucosa, Submaxillary and mesenteric lymph nodes, tonsils.		Rat terrier, intracerebrally.	Remained well for 103 days, when it was killed by the other dogs.
	do	Spinal cord	Normal	Black and tan dog, intracerebrally.	Remained well.
	do	do	do	Rhesus monkey, intracerebrally.	Do.
Dachshund.	Human case in same household (New York City). Dog had paralysis of hind legs of month's duration.	Defibrinated blood.		Rhesus monkey, 15 c. c. intravenously.	Remained well; afterward proved susceptible to poliomyelitis.
Do	do	Defibrinated blood.		Bull pup, 15 cc. intravenously.	Remained well
Do	do	Medulla and cord.	Normal	Dog, intracerebrally and intraperitoneally.	Do.
Do	do	Nasal mucosa.		Dog, intraperitoneally.	Do.
Do	do	Parotid gland and spleen.		do	Do.
Do.	do	5 fleas found at necropsy.		Dog, subcutaneously.	Do.
Irish terrier.	Human case one block away (New York City). Dog became sick, paralyzed, and died in 36 hours.	Medulla and cord.	Slight congestion	Dog, intracerebrally.	Do.
Fox terrier.	Human cases in neighborhood (Newark). This dog had "paralysis and convulsions." Shot.	Spinal cord		do	Do.
Chicken	2 human cases in same household (Elkins, W. Va.). This chicken developed flaccid paralysis of left leg with loss of knee jerks five weeks after human onsets; otherwise normal. 2 other chickens paralyzed and 3 others died after week's indefinite illness, during preceding month.	do	Meningitic congestion and leucocytic infiltration of pia-arachnoid.	Rhesus monkey, intracerebrally.	Do.
Do	do	Spinal cord	As above	Rhesus monkey, intracerebrally.	Do.

SUMMARY.

The uniformly positive results following the inoculation of material from the New York cases are unusual. Their interpretation is difficult. Possibly they signify high virulence of the epidemic; or they may be due to the use of 100 per cent glycerin for preserving the virus. The virus has shown classical infectivity on passage through a series of monkeys, and has been filtered through both Berkefeld and Chamberland filters. Attempts at infection by way of the nasal and alimentary tracts were unsuccessful, though possibly immunity was produced once by the latter method. The neutralization test with this virus was shown to be of questionable value. Infection with the winter strain (West Virginia) of poliomyelitis protected against the 1916 summer strain (New York). The serum of a horse treated with the virus of poliomyelitis, produced no demonstrable immunity in monkeys. None of the paralyses in domestic animals, such as are so frequently reported during an epidemic of poliomyelitis, were shown to have any relation to the disease in people.

III. ATTEMPTS TO INDUCE POLIOMYELITIS IN SMALL LABORATORY ANIMALS.

By A. M. Stimson,

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A series of experiments was undertaken with small laboratory animals—rabbits, guinea pigs, and rats—to determine (1) whether paralytic conditions could be induced in these animals by the introduction of the virus of poliomyelitis, and (2), if this could be done, whether these conditions represented true poliomyelitis or were due to some other cause.

It was conceived that this virus might possibly produce symptoms and lesions in these small animals, which, although specifically due to the organism of poliomyelitis, did not correspond to those classically observed in human beings or monkeys. Hence subexperiments were performed to determine whether or not such deaths as occurred among the inoculated animals were due to the specific virus.

Inasmuch as these experiments resulted negatively, it is considered unnecessary to present complete protocols, but the following summaries are given to show the scope and nature of the experiments.

I. RABBITS.

Four groups of six rabbits each, received material from monkeys which had demonstrated poliomyelitis, a separate monkey furnishing the material for each group. Brain and cord emulsions were used in all animals save four, which received saliva. The inoculations were given intracerebrally, intraperitoneally, intravenously, or by various combinations of these methods. The saliva was given intraperitoneally. A large proportion of the animals in all these groups died, the time of death varying from a day to over two months. Convulsions immediately preceded death in one rabbit, which was found to have a heavy coccidial infestation. Such symptoms have been observed in uninoculated animals. In three instances the animals were found post-mortem to have broken backs. Subinoculations were made in a few instances. In no case in these four groups were symptoms resembling those of classical poliomyelitis observed.

A group of eight animals received intravenous injections of Berkefeld N filtrates of human virus emulsions. After the lapse of 10 days a second dose of freshly filtered material was injected. The virus employed came from four human cases. The dose given varied from 1.5 cc. to 5 cc. No symptoms whatever developed in any of these animals save one, which was found to have a broken back.

The spinal cords of many of the dead rabbits were examined histologically, and in no instance was anything resembling classical poliomyelitis discovered.

II. GUINEA PIGS.

Four groups of six guinea pigs each, were injected with poliomyelitis material from monkeys, all but three animals receiving brain and cord emulsions by combined intracerebral and intraperitoneal methods. The three exceptions received saliva intraperitoneally. The mortality varied greatly in the different groups, two of them escaping with only one death all told, the other two groups losing respectively three and five animals. Only one animal developed discoverable paralysis, and this was found to be due to a traumatic lesion. Histological examinations of spinal cords were uniformly negative as regards poliomyelitis.

III. RATS.

Three groups of six rats each, received poliomyelitis material from three different monkeys, mostly of brain and cord emulsions, into the brain and peritoneum. Six rats received saliva in the peritoneum.

One of these groups escaped with no mortality, except one animal which died after two months; but the mortality in the other groups was nearly complete, occurring in from 3 to 16 days. Subinoculations extending to the fourth remove from the monkey, and involving 34 rats, were made from members of the initial groups. Invariably high death rates resulted. In no instance were paralytic symptoms observed, nor were significant cord lesions discovered in microscopic sections.

An experiment was then undertaken to determine the effect of exposure of brain and cord emulsions of the dying rats to the serum of a recovered poliomyelitic monkey, which serum it was assumed would manifest viruscidal activity. This experiment was controlled by a series of rats receiving untreated emulsion, and one receiving emulsion treated with the serum of a normal monkey. No viruscidal activity was shown by the recovered monkey serum against the infection which was being transmitted in the rats, but control tests on this serum demonstrated it to be inactive also against true poliomyelitis virus when tested upon monkeys.

At the same time with the above experiment a series of rats was injected with the filtrate (Berkefeld N) of the same rat brain and cord emulsion. This filtrate was sterile, according to glucose broth fermentation tube tests. Nevertheless, 5 of 10 rats injected with it died after periods varying from 8 to 41 days.

Four monkeys were injected with brain and cord emulsions from rats which died as the result of the injection of emulsions from other rats, and represented the third remove from the original poliomyelitis virus. Two of these monkeys received repeated subcutaneous injections, and the other two single intracerebral injections. No symptoms developed, and subsequent inoculation with poliomyelitis virus caused the typical disease in these animals.

A series of rats was injected intracerebrally with human poliomyelitis virus emulsion. They all died in from 6 to 14 days without any paralytic symptoms having been noted.

In all of these experiments with rats there was no observed instance of paralysis, nor were histological evidences of poliomyelitis discovered.

DISCUSSION OF EXPERIMENTS SUMMARIZED ABOVE.

In all of these experiments no animal was observed to develop symptoms which correspond in any way to those of poliomyelitis in man and monkeys, except in a few instances where the paralysis was found on section to be due to traumatic or gross inflammatory conditions.

A large number of the animals were sectioned, and in most of them no gross lesions pointing to the cause of death were found. Histological examinations, made on many of the animals, failed to demonstrate lesions even suggesting those of poliomyelitis in man and monkeys.

Many of the animals died during the night without any preliminary symptoms having been observed. They were placed in the ice box as soon as discovered, but opportunity for the migration of bacteria to their central nervous systems was afforded, and smears of many of the spinal cords showed bacterial contamination.

No consistent clinical picture was observed among the sick animals.

While the mortality among the animals injected was in general high, there were marked inconsistencies in the lethality of the various monkey poliomyelitis viruses employed as regards the species into which they were injected. The following example illustrates this fact:

Virus:	Mortality.
M133	Rabbits, high. Guinea pigs, low. Rats, not tried.
M131	Rabbits, high. Guinea pigs, none. Rats, high.
M85	Rabbits, moderate. Guinea pigs, moderate. Rats, low.
M16	Rabbits, high. Guinea pigs, high. Rats, high.

These inconsistencies lead to a strong suspicion that no agent common to the different "strains" used was responsible for the mortality of the different groups of animals. These "strains" were all derived from the same human epidemic at about the same time.

As far as possible, young animals were selected. In one of the rat series, involving 40 animals, the rats were arranged according to size. In general the smaller rats died earlier than the large, but this, it is believed, would have occurred from infections of almost any nature.

The fact that certain of the rats died, following the intracerebral injection of Berkefeld filtrate of rat brain emulsions, can not warrant the conclusion that poliomyelitis virus was present in these emulsions. Another possibility is that some other filterable virus was present. Since fewer rats died from the filtrate injections (50 per cent) than from unfiltered material (77 per cent), some slight support is given to the hypothesis that the deaths among the rats were due to bacterial infections, but it is probable that the mortality in rats following the intracerebral injection of sterile organic matter would be high.

The failure of the rat brain materials, injected subcutaneously, to immunize monkeys against subsequent virulent inoculations has, in the light of present knowledge, no significance. The failure of the intracerebral inoculations of the same material to produce either symptoms or immunity would seem to indicate that the disease in rats was not poliomyelitis.

CONCLUSIONS.

No evidence was adduced by these experiments to show that rabbits, guinea pigs, or rats are susceptible to poliomyelitis.

Other observers have reported the successful infection of rabbits and guinea pigs with poliomyelitis virus. Their methods did not differ essentially from those employed by us. We must therefore attribute the discrepancy to one or the other of two causes. Either the strain of virus employed by these observers differs from ours in its pathogenic properties, or it is contaminated with some organism capable of producing these symptoms in small animals.

IV. REPORT ON ATTEMPTS TO CULTIVATE THE VIRUS OF EPIDEMIC POLIOMYELITIS.

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Of the attempts at cultivation of the virus of epidemic poliomyelitis, those of Flexner and Noguchi seem to have been most consistent. These authors have apparently cultivated a filterable virus, which, from a culture representing the eighth generation, sufficed in virulence to produce the symptoms of the disease in monkeys.

The first work on which this report is based was an attempt to reproduce the above-mentioned results.

Noguchi's technique.—The reproduction of Noguchi's technique was as follows:

Fluid and solid, or semisolid, media made of ascitic fluid or ascitic fluid agar (1 part in 10 parts), to which a piece of sterile tissue was added, were used. The media were used in deep columns in narrow tubes (1 cm. by 20 cm.) covered with a 1½–2½ cm. layer of paraffin oil. Cultures were made by seeding this medium with a piece of spinal cord, or parietal lobe of the brain, from a subject dead of the disease. These cultures were grown at 37° C., within anaerobic and aerobic surroundings, for from 3 to 15 days. The macroscopic criterion of growth was a fine diffuse cloudiness, making its appearance at the bottom of the tube and extending progressively toward the top of the medium, sharply delineating itself within a few centimeters of the surface unless under anaerobic conditions. Microscopic evidence of growth was the presence of minute coccoid or globoid bodies, arranged in groups, pairs, or chains, and staining by the Giemsa or Loeffler's methylene blue stains. Biologic evidence consisted in the monkey test of cultures, sufficiently removed from the original to eliminate the probability of dilution of the virus.

Virus.—The materials used in seeding were cords and brains fresh from monkeys dead of, or prostrated from, the disease, the original strains of virus having come from human subjects dying during the New York epidemic of 1916.

Virus glycerinated for from 14 to 30 days, was also employed in four of the series.

Cultures.—Eighty-three cultures, involving eleven series, were made according to this technique. Sixty-five of the cultures were grown under aerobic conditions, and eighteen under anaerobic conditions in Novy jars. Two control tubes, one without the addition of tissue and one without virus, were made for each series.

Results.—The results were microscopically negative for Noguchi's globoid bodies in all the cultures, and were therefore not tested biologically. In practically all of these cultures, some cloudiness developed and extended upward to varying heights, even to the surface. Not infrequently the same type and intensity of clouding developed in control tubes.

Sixteen cultures (or about 20 per cent) showed diphtheroid bacteria in the cloud or sediment.

Four (or 5 per cent) showed cocci, and ten (or 12.5 per cent) showed small coccoid or granular bodies, staining a violet to blue color with the Giemsa or Leishman stain. These bodies were found, however, in three controls; and similar bodies could be reproduced in the staining of other albuminous films.

Modifications of Noguchi's technique.—This technique was modified in several ways, by employing columns of media without an oil cap; tubes slightly larger in diameter; ground tissue, instead of blocks of tissue; media of altered reaction; and albuminous media other than ascitic fluid.

There were no striking results obtained by the use of any of these modifications. Tubes of 18 by 150 mm. were used in all the tests other than the above-mentioned 83 cultures.

Employing cultures without oil cap, in deep column, and aerobic surroundings, two series of four cultures each were made. One virus was represented.

Results.—Granular bodies in 50 per cent; Diphtheroids or cocci in 12 per cent. Granular bodies in one of four controls, and cocci and ghosts of bacteria in another; two controls negative.

Series of cultures were made covering one or more modifications; as for example: series including ground virus and ground tissue, as opposed to blocks of virus and blocks of tissue; block virus with ground tissue, or ground virus with block tissue; the media and methods remaining the same in a comparative series, but varied in covering several series, so as to include the use of other albuminous media. Whey, hydrocele fluid, and samples of ascitic fluid from several sources were used, also whey to which hydrocele or ascitic fluid was added.

All of these cultures were incubated under oil caps, some in Novy jars in an hydrogen atmosphere, others under aerobic surroundings. Rabbit kidney, guinea pig kidney, and monkey kidney, rabbit brain, and egg yolk were severally used as the tissue addition.

The whey was prepared by coagulation with rennet and filtering through paper and the Berkefeld, using it raw, and by filtering through paper and sterilizing in the Arnold.

These modifications were covered in 17 series of tests, involving 165 cultures.

Results.—Diplococci and streptococci were found in 11 of 28 cultures in which the raw Berkefeld filtered whey, or sterilized whey, was used as a medium or was combined with hydrocele or ascitic fluid as a medium.

In the entire number of cultures, cocci in clumps were found five times (about 3 per cent); diplococci and streptococci, 17 times (about 10 per cent); diphtheroids, 12 times (about 7 per cent); granular bodies, 27 times (about 16 per cent). Granular bodies were found in about 10 per cent of the controls in these series.

The findings of granular bodies in controls without virus, as well as in the cultures including virus, led us to ignore them in subsequent tests.

Solid media.—Three series of ascites tissue agar, or ascites dextrose tissue agar tubes, comprising 35 shake cultures, were seeded with virus, but the entire series was discarded because of the development of macroscopic colonies of cocci or diphtheroids.

In following out the methods of Noguchi a number of check tests were made, involving the use of fluid and solid media by routine methods.

Fermentation tubes were used, containing ascites fluid and tissue, ascites fluid tissue dextrose broth, ascites fluid tissue plain broth, tissue glucose broth, egg yolk glucose broth, and tissue galactose peptone water, involving 40 cultures.

Solid slants of coagulated egg yolk and plates of blood agar were incubated in Novy jars in an atmosphere of hydrogen or carbon dioxide, and in partial oxygen tension but, in the absence of any specific findings, were not detailed in the record.

In several of these routine cultures diphtheroids and cocci were found.

All cultures in which bacteria were found were regarded as contaminated and discarded.

Rosenow's technique.—With the finding, by Rosenow, of polymorphic streptococci in ascites dextrose media to which virus was added, attempts were made to duplicate his results.

The technique adopted was similar to that of Noguchi, excepting the composition of the media. The fluid medium was a dextrose bouillon base; dextrose 0.2 per cent, beef extract, peptone broth; reaction +0.6 to phenolphthalein titration; ascitic fluid added 1 part in 10 parts of medium.

Later the technique was modified by the use of "mass" cultures, grown in nursing bottles with straight sides, of the "Klean-easy" type, in which ½ ounce of ascitic fluid was added to 4½ ounces of the dextrose broth. Incubation was at 35° C. A very much larger amount of virus was seeded, both with and without the presence of other tissue, and the cultures were examined and used when not over 24 hours old. Whey of a +0.6 reaction was also used as a medium, both alone and in combination with ascitic fluid.

The identification of the organism was by its morphology, and by the production of minute green aerobic colonies on rabbit-blood agar plates (rabbit blood 1 part, dextrose agar 20 parts).

Animal tests.—The attempts to reproduce the disease in animals were made in rabbits, guinea pigs, puppies, white rats, and monkeys. In the earlier work rabbits weighing 1,200 to 1,800 grams were used, but later younger animals were used: rabbits of 400 to 900 grams, guinea pigs of 100 to 200 grams, and white rats of 40 grams.

Routes of inoculation.—Inoculations were made by intravenous, intracerebral, intraperitoneal, and subcutaneous routes; in monkeys, also by intraspinal routes.

Dosage.—The dosage of culture used varied from 0.5 cc. to 2 cc. (dependent on weight) of an emulsion made by centrifuging from 15 to 60 cc. of a 24-hour fluid culture, and taking up the sediment in 10 cc. of salt solution, or of the supernatant fluid.

Virus.—Fresh and glycerinated virus of monkey and of human origin were treated by this method; also tonsils from a human case of the disease.

Tests.—Twenty-one series were made, using dextrose media, according to the technique followed by Noguchi, and by Rosenow. Eighteen fresh viruses, two glycerinated, and one pair of tonsils from a clinically and histologically proven human case of epidemic poliomyelitis were used.

Results.—These series included 208 primary cultures, in which diplo- and streptococci were found in 28 cultures (13 per cent); cocci in 15 (7 per cent); diphtheroids in 49 (23 per cent).

Many of these findings overlap, in that more than one of the above types of organism were found in the same tube.

Subcultures were made in the deep-fluid cultures, and on rabbit-blood agar plates, for purification and identification of the organisms recovered.

To test the organisms found in the cultures and subcultures, 168 guinea pigs, 70 rabbits, 9 rats, 5 puppies, and 9 monkeys were used.

In none of these animals were there any histological findings suggestive of poliomyelitis.

In two of six animals, one rabbit and one guinea pig, injected intravenously with cultures from the medulla, and in one of three rabbits injected intravenously with cultures of the tonsils of a human case of poliomyelitis there were symptoms of meningeal involvement, including spastic paralysis and convulsions. On section there were no macroscopic findings in the central nervous system other than some congestion and slight increase in the cerebrospinal fluid. Smears from this fluid were filled with diplo and streptococci, many of which were engulfed in polymorphonuclear leucocytes.

Cultures from the meninges of these animals, inoculated intravenously into three guinea pigs, produced similar findings in one of the three in three days. Two subsequent series, each made from the previous set of animals, showed similar lesions.

Two monkeys injected with these cultures intravenously, developed a general septicemia, and two injected into the subarachnoid spaces developed meningitis, but not histological poliomyelitis.

Tests of ascitic fluid.—Four lots of ascitic fluid were tested. The test was made by adding 15 cc. of each lot of ascitic fluid to 125 cc. of dextrose broth in a nursing bottle. These nursing bottles were incubated at 35° C. for 72 hours. Microscopic examination of the cultures was negative.

A second test was made in which tissue, autoclaved in the broth, was added to the dextrose broth. In this test three of the four cultures were contaminated. A diphtheroid organism was found in one of the cultures, and small pleomorphic streptococci were found in the other two cultures.

Another series of tests was made of eight lots of ascitic fluid. Four of these lots were represented in the previous tests, but in this series other samples than those cultured were used.

Five samples were shown to be contaminated, when grown in the dextrose tissue broth, but were sterile when grown for one week in dextrose broth without tissue.

The tissue was autoclaved in the broth, to avoid the possibility of the contamination being introduced by the tissue.

These five samples represented four lots of ascitic fluid, or one additional to those already found contaminated.

SUMMARY.

Numerous diplococci and streptococci have been cultivated from the spinal cords and brains of human and monkey subjects which showed, clinically and histologically, the findings in acute epidemic poliomyelitis.

None of the organisms cultivated reacted in the small laboratory animals nor in the monkeys with clinical or histological evidence of the disease.

The attempt to reproduce Noguchi's and Rosenow's results were negative, though involving approximately 700 primary cultures and 300 animal tests.

DISCUSSION.

In view of the not infrequent reports in the literature for many years of myelitis and poliomyelitis experimentally produced by the injection of streptococci isolated from various sources, it is remarkable that none were found among these experiments.

The negative findings in attempts to reproduce the results of the above-mentioned workers, though carrying out their technique as guided by their writings, would suggest that the virus used did not contain organisms similar to those found by Noguchi or Rosenow, or that the organisms were of unusual sensitivity, and were lost by imperceptibly slight modifications made of the technique.

Diplococci and streptococci were found in several samples of ascitic fluid, when cultures were made under a lowered oxygen tension. Therefore the presence of such organisms in cultures, to which has been added raw ascitic fluid, should not be misinterpreted. The organisms are frequently present in small numbers, and may require the development of several days' incubation before they become evident.

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HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

†No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

*No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygenic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

†No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi.* By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitos (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

*No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

*No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

†No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

†No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

*No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

*No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lypolytic hydrolysis of ethereal salts. By J. H. Kastle.

*No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

†No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

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TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY—BULLETIN No. 112

APRIL, 1918

I. PHENOLS AS PRESERVATIVES OF ANTIPNEUMOCOCCIC SERUM. A PHARMACOLOGICAL STUDY

By CARL VOEGTLIN

II. THE NATURE OF CONTAMINATIONS OF BIOLOGICAL PRODUCTS

By I. A. BENGTSON

III. STUDIES IN PRESERVATIVES OF BIOLOGICAL PRODUCTS. THE EFFECTS OF CERTAIN SUBSTANCES ON ORGANISMS FOUND IN BIOLOGICAL PRODUCTS

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PHENOLS AS PRESERVATIVES OF ANTIPNEUMOCOCCIC SERUM. A PHARMACOLOGICAL STUDY.

By CARL VOEGTLIN, Professor of Pharmacology, Hygienic Laboratory, United States Public Health Service, Washington, D. C.

INTRODUCTION.

From recent reports on the serum treatment of acute lobar pneumonia it would appear that this treatment, if applied judiciously to selected cases, may benefit many patients. For instance, Cole (1917) states that his experience with a specific serum, obtained by treating horses with dead cultures of Pneumococcus Type I, leads him to believe in the therapeutic value of this serum in the treatment of cases of pneumonia due to this particular microorganism.

Similar experiences with this same serum were had by various other clinicians. These reports will probably result in a more widespread use of antipneumococcic serum. As a matter of fact, several manufacturers of biological products have already placed such a serum on the market.

The purpose of this paper is to report a number of pharmacological experiments which were made in order to determine the suitability of trikresol and phenol as preservatives for antipneumococcic serum. The work was taken up for the following reasons: (1) Most manufacturers of serums resort to the addition of trikresol or phenol to serums, in order to preserve their products from bacterial contamination. For this purpose from 0.3 to 0.5 per cent of phenols are added to the serums. (2) The advocates of antipneumococcic serum recommend the use of large individual doses (50 to 100 c. c.) given intravenously at intervals of a few (6 to 8) hours. This means that the patient may receive with a single injection as much as 0.5 gm. of phenols directly into the blood stream and, with repeated injections, from 1.2 to 2 gm. in the 24 hours. Inasmuch as phenols are fairly toxic substances, it becomes a matter of importance to determine whether or not the amount of phenols administered with the antipneumococcic serum may give rise to any undesirable symptoms and, if this should be the case, how to avoid them.[1] It was therefore

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[1] For literature and experiments on the pharmacologic action of serum preservatives, see Voegtlin, Hyg. Lab. Bull. 96.

2.35 p. m. The blood pressure was 110 mm. and spontaneous respiration had reappeared. Artificial respiration was discontinued. Blood pressure gradually fell to 40 mm. at 2.39 p. m. Heart stopped beating. Respiration had failed at 2.38 p. m.

Artificial respiration again led to recovery of blood pressure, and partial recovery of spontaneous respiration.

Experiment 3.—Dog, 11.3 kg. Received at 1.00 p. m., 11.3 c. c. chloretone solution (= 3.4 gm.) by stomach tube.

1.43 p. m. Blood pressure 102. Injection of 8 c. c. 0.5 per cent trikresol Locke solution in 1 minute and 12 seconds (see tracing 4).

Blood pressure at end of injection 70 mm.

Respiration temporarily slowed (from 42 to 34 per minute).

1.49 p. m. Blood pressure 76.

1.50 p. m. Blood pressure 92.

1.51 p. m. Blood pressure 106.

1.53 p. m. Blood pressure 110.

1.58 p. m. Blood pressure 108. Injection from burette of 22.6 c. c. 0.5 per cent trikresol serum. Injection finished at 2.03 p. m. (see tracing 10). During the injection the blood pressure fell from 108 to 98 mm. The respiration, which was 36 per minute previous to the injection, rose gradually to 56 per minute.

2.25 p. m. Blood pressure 106. Rapid injection, by means of syringe, of 10 c. c. 0. 5 per cent trikresol serum (injection time 30 seconds). Blood pressure first rose 6 mm. above previous level, then fell to 104.

2.27 p. m. Blood pressure 110.

2.51 to 2.55 p. m. Blood pressure 116. *Slow* injection of 22.6 c. c. 0.5 per cent trikresol Locke solution. There was practically no change, in blood pressure or respiratory tracing, following the injection.

Experiment 4.—Monkey, 3.7 kg. Received at 2.08 p. m., a large dose (2.5 gm.) of chloretone, dissolved in olive oil, intraperitoneally. The animal gradually became deeply anesthetized. The respiratory and blood pressure tracings showed that the circulation and respiration were very much depressed. The heart showed irregularities; the respiratory movements were irregular.

3.41 p. m. Intravenous injection of 0.5 per cent trikresol serum by means of syringe. This was followed by a fall in blood pressure from 54 to 40 mm., with a slow recovery. The respiratory movements temporarily became deeper and more regular (see tracing 11).

3.50 p. m. The blood pressure and respiration were much depressed. Intravenous injection of 10 c. c. normal horse serum. This caused a further depression of the respiratory movements and a fall in blood pressure. A few minutes later artificial respiration was begun. This resulted in a rise of the blood pressure to the level attained previous to the last injection (see tracing 12).

4.12 p. m. Blood pressure 46 mm. Cardiac irregularities were still present, although not so pronounced as at the time before artificial respiration was begun. Intravenous injection of 10 c. c. of 0.5 per cent trikresol serum. Blood pressure fell to 34 mm. and gradually rose to 46 mm. within 10 minutes.

Experiment 5.—Dog, 2.7 kg. Received at 9 a. m. 30 c. c. of a four-hour ascitic broth culture of Type I Pneumococcus by means of an intrabronchial tube.

3 p. m. Animal had sick appearance. Rectal temperature 41° C. Respiration labored, 52 per minute. Pulse 120.

3.40 p. b. Condition unchanged. Dog received 2.4 gm. chloretone, dissolved in olive oil, intraperitoneally.

4.05 p. m. Intravenous injection of 6.6 c. c. antipneumococcic serum containing 0.4 per cent of trikresol (burette). Practically no change in blood pressure or respiration.

4.10 p. m. Second injection of same serum, 10 c. c. Slight fall in blood pressure (from 88 to 84 mm.). No change of respiratory movements.

4.14 p. m. Injection of 10 c. c. 0.4 per cent trikresol horse serum (see tracing 8). Blood pressure rose temporarily from 90 to 102 mm. (12 mm.), to return to previous level one minute later. The respiratory movements were not influenced by injection.

4.18 p. m. Injection of 10 c. c. antipneumococcic serum (same as above). Neither blood pressure nor respiratory tracings were influenced.

4.21 p. m. Injection of 10 c. c. 0.4 per cent trikresol horse serum. Blood pressure rose 12 mm. during injection. Respiratory movements not influenced.

4.25 p. m. Respiratory movements gradually became deeper.

4.33 p. m. Injection of 10 c. c. 0.4 per cent trikresol Ringer solution (see tracing 9). Blood pressure fell 6 mm. after the injection was finished. Recovery to previous level after 10 minutes. The respiratory movements at end of injection decreased in depth for about 8 minutes.

5.10 p. m. The dog was killed with chloroform. No evidence of failure of the respiration was observed during the entire experiment; the animal would probably have lived for a considerable length of time.

Necropsy: Red hepatization of lower left, and part of lower right, lobe of lung.

DISCUSSION.

Effect of intravenous injections of normal horse serum.—Intravenous injections of quantities up to 3 c. c. per kilo body weight, of normal horse serum without preservative, as a rule produced a moderate and temporary rise in blood pressure, even when the rate of injection was relatively slow (see tracing 1). Similar injections of normal saline solution did not produce a rise in blood pressure. From this it would appear that normal horse serum contains a pressor substance, a view which has been repeatedly expressed by various investigators (O'Connor, 1911, Voegtlin and Macht, 1913).

Effect of intravenous injections of Ringer's or Locke's solution containing 0.4 to 0.5 per cent trikresol or phenol.—A rapid injection of Ringer or Locke solution, containing from 0.4 to 0.5 per cent trikresol or phenol, produces a typical phenol effect on blood pressure, which is characterized by a rapid fall and a gradual recovery within a few minutes. This action of the phenols on the blood pressure is due, according to Pilcher and Sollmann (1915), mainly to the depressing action of the drug on the heart, although the depression of the vasomotor center may constitute a contributing factor. The respiratory movements are influenced by the phenol only to a minor extent. The factor which decides the degree of blood pressure fall is undoubtedly the rate of injection. Thus, it was observed that, with a very slow rate of injection, relatively large amounts of phenol-Ringer solution could be injected intravenously without causing more than a trivial fall of blood pressure. The respiratory movements were sometimes decreased in depth and rate, this being, however, only a fleeting effect.

Effect of intravenous injections of normal horse serum containing 0.4 to 0.5 per cent phenol or trikresol.—The result of the injection of this serum was not always the same in the various animals. When the circulation was in good condition (light anesthesia), the injection of the serum at a moderate rate produced a moderate rise in blood pressure (see tracings 1a and 8). With a depressed circulation (overdose of anesthetic), the injection of the serum nearly always produced a fall of a few millimeters in blood pressure (see tracing 5). It seems as though, in the first instance, the vascular apparatus responds mainly to the pressor properties of the serum; whereas, in the second case, the depressing action of the phenol overbalances the pressor action of the serum *per se.*

In one experiment (see tracing 11), in a monkey whose respiration was about to fail on account of an overdose of chloretone, the injection of trikresol serum was followed by a temporary improvement in the respiratory movements. Whether or not this change of respiration was due to the trikresol can not be definitely stated. It may suffice to refer to observations of Auer (1915), who found that, at times, the respiratory center is stimulated by trikresol.

That phenol serum does not produce characteristic phenol effects on the blood pressure may be due to the probability of the phenol entering into chemical combination with the serum proteins (Cooper, 1913). It is furthermore conceivable that the phenols of the phenol serum are in part dissolved in the serum lipoids; a fact which would naturally reduce the concentration of free phenols in the blood stream immediately following an injection of phenol serum.

Effect of old antipneumococcic serum preserved with 0.4 per cent trikresol.—The action of this serum differed from that of freshly prepared 0.4 per cent trikresol serum, inasmuch as the pressor effect of the former was much less pronounced. As a matter of fact, we observed, nearly always, a fall in blood pressure following the injection of this antipneumococcic serum. It is perhaps possible to interpret these findings by assuming that the pressor properties of the blood serum are lost in time, either as a result of the action of the phenol preservative on the pressor substance or for other reasons (oxidation of epinephrine contained in serum).

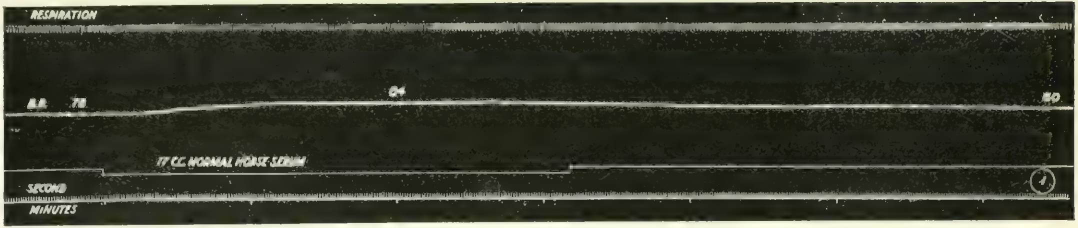
Observations made on a dog with lobar pneumonia.—It is altogether impossible to draw final conclusions from a single experiment of this kind. It should be pointed out, however, that the results obtained with the various serums and phenol solutions were of the same character as those obtained on the other animals (see tracings 8 and 9).

As will be seen from the previous discussion, the addition of a moderate amount of trikresol, or phenol, to serum does not impart to the serum properties which might lead to alarming symptoms in animals receiving intravenous injections of such serums. Even with doses twice as large (per bodyweight) as those used in the practice of the serum treatment of lobar pneumonia in the human, no untoward effects were observed. Moreover, the repetition of these injections, at intervals of about 10 to 20 minutes, does not result in symptoms which might in any way be called alarming. It is, perhaps, superfluous to state that we never observed toxic symptoms such as muscular twitchings, convulsions, or collapse in any one of the animals of this series.

In spite of the fact that phenol serum causes only moderate changes in blood pressure and respiration in animals the possibility of its giving rise to more serious symptoms in patients with pneumonia is still left open, although such a possibility is rather remote. Relatively little is known concerning the pathological physiology of lobar pneumonia. Clinical, as well as experimental, studies have brought out the fact that the circulation is abnormal. Recent studies on experimental pneumonia, by Newburgh, Means, and Porter, 1916, point to an abnormal condition of the respiratory center in the later stages of the disease. It was shown by these investigators that the response of the respiratory center to increasing concentrations of carbon dioxide is much decreased. The center seems to be in an exhausted state, as a result of an excessive stimulus of unknown origin arising in the diseased lung tissue and affecting the center by way of the vagi. That the functions of the heart and respiration are abnormal in pneumonia is perfectly evident from even superficial observation. Under these conditions, it might be possible that the phenol preservatives of administered serum might cause a collapse of either cardiac or respiratory activity. However, this is very unlikely to occur, provided certain precautions are observed in the administration of the serum. It should be strongly emphasized that the rate of injection should be as slow as possible (see also Cole, 1917).

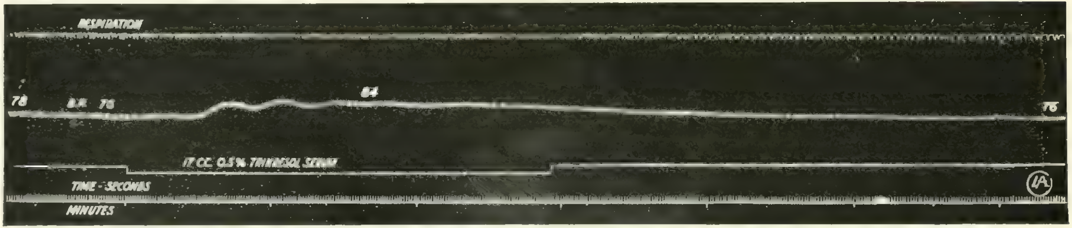
So far, only the immediate effect following the injection of phenol serum has been considered. It may justly be asked if such injections might not lead to more remote toxic effects. This question did not seem worthy of investigation for the following reasons: It is well known that small doses of phenols introduced into the animal body, either by way of the stomach or directly into the blood stream, rapidly disappear from the blood. The fate of phenols in the body has been studied extensively, and it has been demonstrated that these substances are rapidly excreted, mainly by the kidney, and in traces by the lungs and sweat glands. Part of the phenols are transformed in the body into products of lesser toxicity, namely, conjugated sulphates and glycuronates. Another part of the phenols is more or less completely oxidized (for literature see Folin and Denis, 1915). Furthermore, there is considerable experimental and clinical evidence to the effect that relatively large quantities of phenols can be taken up by the animal body, without causing more than mild symptoms of poisoning. Thus, it was shown, in the days following the introduction of the carbolic spray by Lister, that many surgeons could expose themselves to the prolonged action of phenol for years without suffering from poisoning. Of course, some cases of phenol poisoning were reported as a result of this practice, but the majority of surgeons escaped.

Hence it is very doubtful whether the phenols, administered together with serum to patients with lobar pneumonia, would cause even remote symptoms of phenol poisoning. In spite of this conclusion, it is desirable to take into consideration every possible precaution. For instance, it might be inadvisable to give serum preserved with phenols to patients with nephritic complications, since phenols are known to have an injurious action on the kidneys, as evidenced by the appearance of albumen in the urine after the injection of phenol.



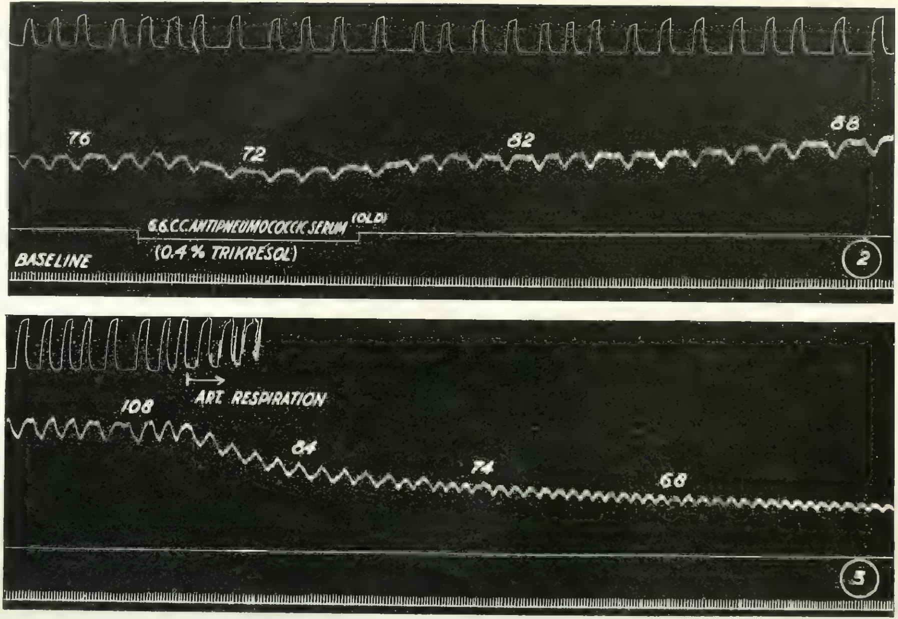
TRACING 1.—EXPERIMENT 1.—DOG, 9.5 Kg.

Shows effect of injection of normal horse serum. Note temporary slight rise in blood pressure.



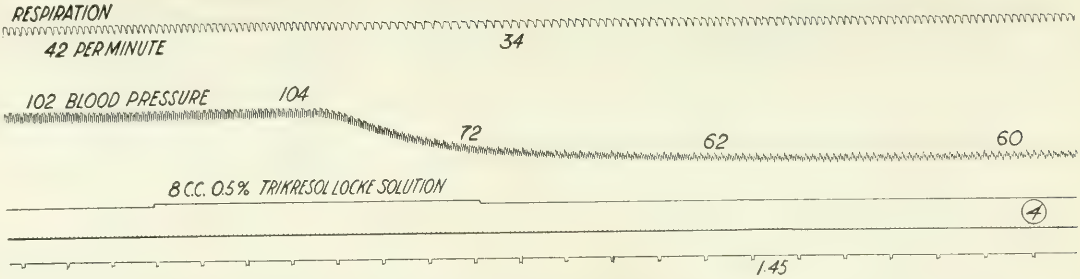
TRACING 1A.—EXPERIMENT 1.

Shows effect of injection of 0.5 per cent trikresol serum. Note temporary slight rise in blood pressure.



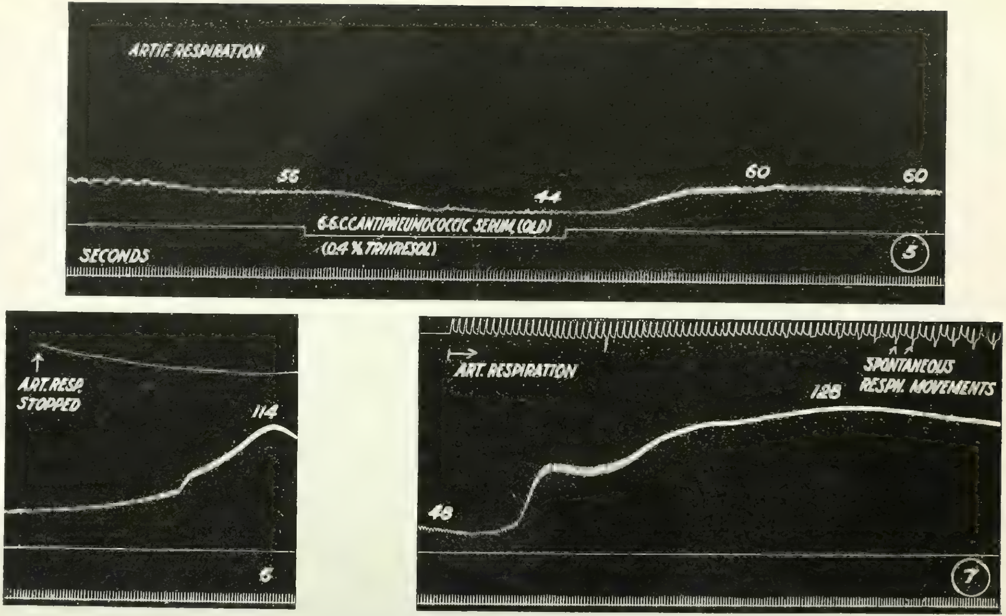
TRACINGS 2 AND 3.—EXPERIMENT 2.—CAT, 2 Kg.

Shows slight fall in blood pressure as a result of injection of 6.6 cc. antipneumococcic serum, containing 0.4 per cent trikresol. After the injection the blood pressure rises and respiration deepens. Note fall in blood pressure with beginning of artificial respiration. Rise in blood pressure following injection was probably due to partial asphyxia from an overdose of chloretone.



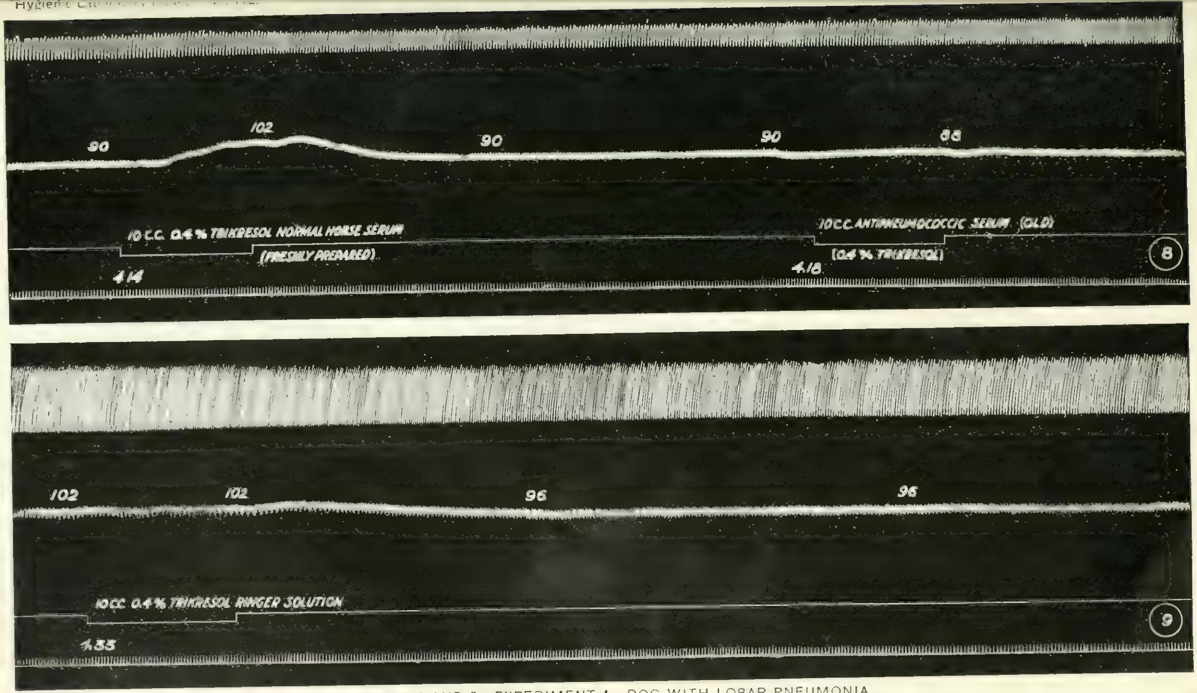
TRACING 4.—EXPERIMENT 3.—DOG, 11.3 Kg.

Shows marked fall of blood pressure produced by injection of 8 cc. 0.5 per cent trikresol-Locke solution. Respiration is temporarily slowed.



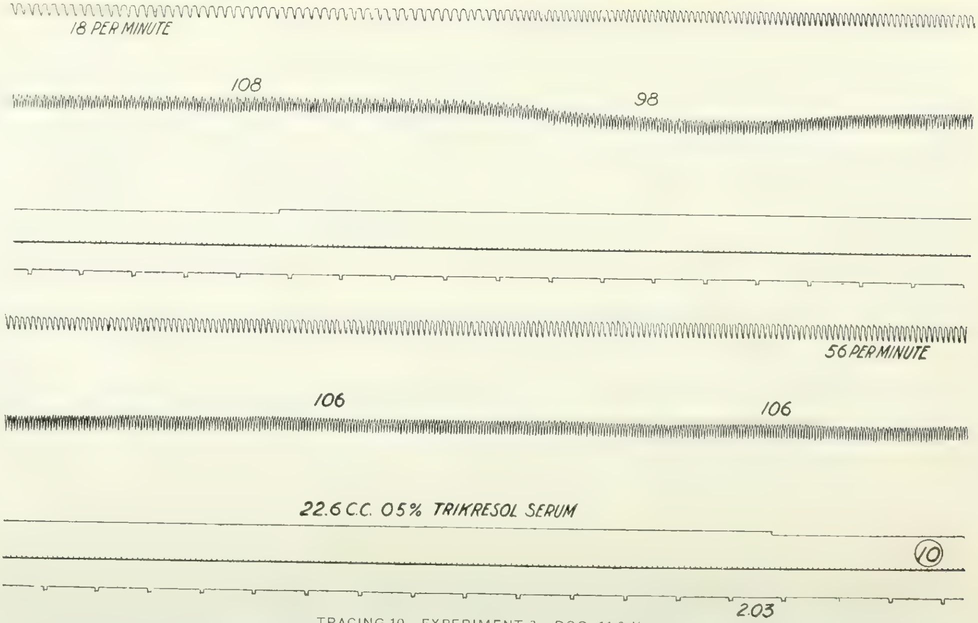
TRACINGS 5, 6, AND 7.—EXPERIMENT 2 (continued).

Tracing 5 shows fall of blood pressure following injection of antipneumococcic serum.
Tracing 6 shows asphyxial rise in blood pressure due to interruption of artificial respiration.
Tracing 7 shows recovery of blood pressure with artificial respiration. These tracings show that in spite of an overdose of the anaesthetic the serum preservative causes only a temporary depression of the circulation.



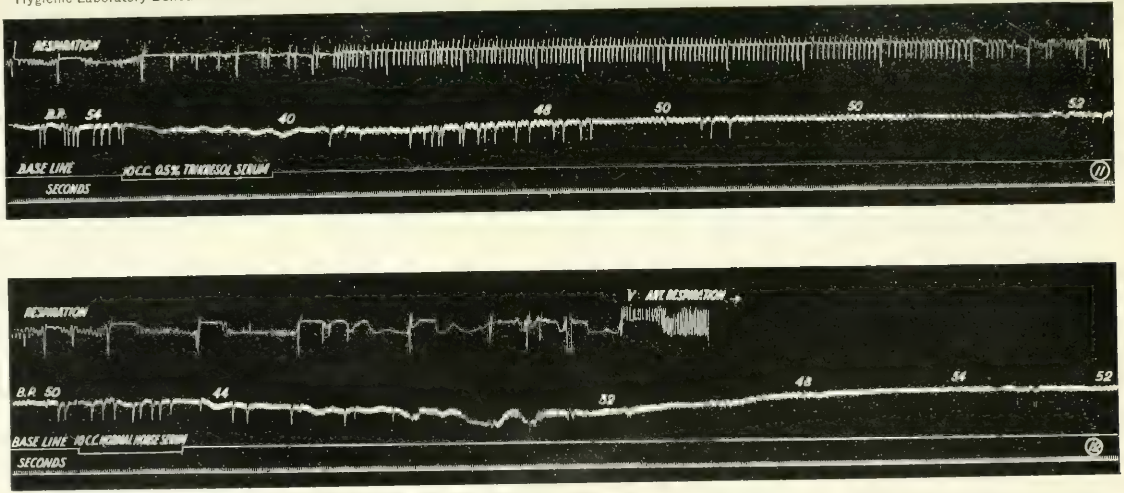
TRACINGS 8 AND 9.—EXPERIMENT 4.—DOG WITH LOBAR PNEUMONIA.

Tracing 8 shows depressor effect of 0.4 per cent trikresol serum (freshly prepared) and slight depressor effect of old antipneumococcic serum containing 0.4 per cent trikresol.
Tracing 9 shows depressor effect of 0.4 per cent trikresol Locke solution on both respiration and blood pressure.



TRACING 10.—EXPERIMENT 3.—DOG, 11.3 Kg.

Shows insignificant effect on blood pressure of a slow injection of 22.6 cc. 0.5 per cent trikresol serum. Note also increase in respiratory rate and depth. This quantity of trikresol serum is about twice as much, calculated per bodyweight, as a patient would ordinarily receive even with a maximum dose of antipneumococcic serum.



TRACINGS 11 AND 12.—EXPERIMENT 4.—MONKEY, 3.7 Kg.

Tracing 11 shows depressor action of trikresol serum on blood pressure in an animal with marked depression of the circulation and respiration due to an overdose of chloretone. Previous to the injection the heart action was very irregular and the respiration was about to fail. Note temporary improvement in respiratory movement and cardiac activity.

Tracing 12 is a continuation of Tracing 11 and shows effect of injection of normal serum. Note improvement of blood pressure as a result of artificial respiration.

These cases are probably not very numerous and cautious procedure would undoubtedly avoid any serious consequences.

CONCLUSIONS.

The addition of trikresol or phenol to antipneumococcic serum in concentrations not exceeding 0.5 per cent does not seem to impart to this serum toxic properties which might give rise to serious symptoms in the serum treatment of pneumonia.

On the basis of the observations recorded in this paper, it is concluded that the intravenous injection of antipneumococcic serum should be made at a very slow rate.

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THE NATURE OF CONTAMINATIONS OF BIOLOGICAL PRODUCTS.

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In the testing of biological products for sterility contaminations are sometimes encountered. These may be actually in the product, or may occur as the result of incomplete sterilization of the medium used in making the tests, or by accidental contamination in the process of testing.

The method used in this laboratory in making sterility tests of the various biological products consists in planting with a Pasteur pipette, with aseptic precautions, 5 and 20 drop amounts of the product into 2 sterile dextrose broth Smith fermentation tubes, and incubating these at 37° C. for seven days. If growth occurs in either or both of these tubes within the seven-day period, a replant of the same product is made into six fresh dextrose broth tubes, three with 5-drop amounts and three with 20-drop amounts. If no growth occurs in the replant, it is assumed that the contamination was accidental. If growth occurs, however, and, if by miscroscopic examination the same organism is found as was present in the first planting, the contamination is considered to have been in the product. In the case of products contained in small ampoules, which have only a sufficient amount to make one planting, a number of fresh ampoules are planted a second time, if growth occurs in any of the first lot planted.

It is not always possible to determine with certainty whether the organism found was in the product or was accidentally introduced in the planting, particularly when only a few growths are obtained among a large number of containers planted. In the filling of ampoules, the product in bulk may have been sterile and single ampoules accidentally contaminated during the process of filling. In such cases a large number of ampoules must be planted in order to arrive at any definite conclusion in regard to the nature of the contamination.

Contamination of the media used for planting due to incomplete sterilization or to wetting of cotton plugs through which contamination might enter after sterilization has been practically eliminated in the testing by incubating the broth used in making the tests for a period of seven days previous to planting.

The organisms which were judged to have been contaminations actually in the products tested during the period from November, 1914, to January, 1917, have been retained, and during the latter part of this period cultures of organisms which were obtained as the result of accidental contamination were also saved.

Those considered to be in the products were secured from specimens forwarded by manufacturers or boards of health for check testing, from experimental lots of products made at this laboratory, and from samples secured at the inspection of manufacturing establishments or secured by purchase in the open market. In this way over 400 cultures were collected. In the present paper the greater part of the organisms which were considered to be contaminations in the products (consisting of about one-fifth of the total number, which includes some duplicates) have been studied, with a view to ascertaining the nature and the frequency of occurrence of the different forms.

The organisms studied have been grouped and considered under the following headings:

1. Coccaceæ.
    - *a.* Cells in irregular groups.
    - *b.* Cells in chains.
2. Non-spore-bearing rods.
3. Spore-bearing rods.
    - *a.* Aerobic.
    - *b.* Anaerobic.

In all cases the cultures were plated out and characteristic colonies fished in order to obtain pure cultures.

1. COCCACEÆ.

(a) *Cells in irregular groups.*—The plan followed in the study of the *Coccaceæ* is outlined below, attention being paid to the pathogenicity of the various organisms considered and to the cultural and other characteristics which might be correlated with pathogenic properties. The cultural study of this group is based on the work of Winslow and Winslow, entitled "The systematic relationship of the *Coccaceæ*,"[1] with such modifications as seemed necessary in view of the fact that organisms from a particular source were being considered.

1. Morphology.
2. Gram stain.
3. Vigor of growth on slant agar.
4. Pigment production.
5. Behavior in broth, litmus, milk, and gelatin.
6. Hemolysis.
7. Reduction of nitrates.

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[1] The systematic relationships of the *Coccaceæ* . . . , by C.-E. A. Winslow and A. R. Winslow. N. Y., J. Wiley & Sons, 1908.

8. H-ion concentration (dextrose-phosphate-peptone medium).
9. Thermal death point.
10. Pathogenicity.

METHODS.

Morphology.—Morphology was determined from smears made from 24-hour-old agar cultures stained for 1 minute with aqueous methyl violet stain.

Gram stain.—The Gram stain was applied as follows, 24-hour cultures from agar slants being used for smears: (1) stained with methyl violet, 1 minute; (2) Gram stain, 1 minute; (3) washed with absolute alcohol, the slide being held in an inclined position so that the alcohol could drip off the edge, the process being continued until the washings showed no color; (4) rinsed in tap water; (5) counterstained with a $\frac{1}{10}$ per cent dilution of carbol fuchsin, 10 seconds; (6) rinsed in tap water.

Growth on agar.—Growth on agar was observed after 14 days at 37° C. Pigment production was determined from the cultures grown at 20° C. for 14 days.

Broth.—Nine cultures were planted in broth and appearance of growth recorded at the end of 3 days and 7 days. As this test seemed to be of no particular value for differentiation in this group of cultures, it was discontinued.

Litmus milk.—The behavior in litmus milk was recorded in 24 hours and at the end of 7 days and 14 days.

Gelatin.—The determinations of liquefaction of gelatin were made by inoculating the culture on the surface of the gelatin and measuring the amount of liquefaction in millimeters after 30 days incubation at 20° C.

Hemolysis.—Hemolysis was tested for on blood agar plates, the plates being prepared as follows: About 5 c. c. of defibrinated rabbit's blood was added to 100 c. c. of melted agar (0.5 per cent NaCl) previously cooled to 45° C, and plates poured, allowing about 6 c. c. to each plate. The plates were streaked with 24-hour broth cultures of the various organisms and incubated at 37° C. Readings were made after 24 hours.

Reduction of nitrates.—Reduction of nitrates was tested for by incubating duplicate tubes of nitrate medium (1 gm. peptone, 0.2 gm. nitrite-free potassium nitrate, in 1000 c. c. distilled water) for 7 days at 20° C. and making the test for nitrites by the use of the reagents sulphanilic acid and a-amidonaphthalene acetate solution, made up according to the Standard Methods of the American Public Health Association. Two c. c. of cultures were used and 1 c. c. of each of the reagents. Ammonia was tested for by the use of Nessler's reagent in the same medium.

Hydrogen ion concentration.—Instead of using the method of titration of acid produced from dextrose and lactose and other carbohydrates and alcohols, which method has heretofore been extensively used in the classification of Staphylococci and Streptococci, the H-ion concentration in a special medium containing dextrose was studied with the hope that some relation might be found between the H-ion concentration and the degree of pathogenicity. The medium used contained 1 per cent of Witte's peptone, 1 per cent of dextrose, and 1 per cent of dibasic potassium phosphate.

Thermal death point.—The thermal death point was determined by the following method:

The cultures were grown on agar slants, 24 hour old cultures being used in making the test. The growth was suspended in sterile salt solution and after shaking and allowing clumps to settle, a portion was drawn up into a Pasteur pipette, of not more than 1 mm. bore. A column about 2 inches in height was drawn into the pipette, the lower end was sealed off in the flame and the pipettes immersed in a water bath in an upright position, and allowed to remain at the desired temperature for half an hour. Duplicate tests were made in each case. After heating, the contents of the pipettes were planted in dextrose broth and growth noted.

TABLE I.

No.	Product from which isolated.	Morphology.	Gram stain.	Agar.	Pigment.	Broth.	Litmus milk.	Gelatin (30 da.).	Hemolysis	Reduction of nitrates.		H-ion concentration.	Thermal death point.	Pathogenicity.
		Diam.								Nitrites.	NH³.			
1010	Tuberculin	Coccus .8μ	Positive.	Heavy, raised, spreading growth	White	Turbid ++, no scum or ppt.	Alk. (7 da.) A ? (14 da.).	No liq	+(24 hr.)	Trace.	Neg.	pH5.0	57½° C. (½ hr.)	Nonpathogenic for rabbits and mice.
1011	do	Coccus .7μ	do	do	do	do	Alk(?) 14 da	do	Neg	Neg.	Neg.	5.0	60° C. (½ hr.)	Do.
1014	Bacterial vaccine.	Coccus .8μ	do	do	do	Turbid +, no scum or ppt.	A+ (2 da.) A +++ (14 da.).	Liq. 8 mm	do	Neg.	Trace	6.0	57½° C. (½ hr.)	Do.
1016	Bacterial extract.	Coccus .8μ	do	do	Colorless.	do	Alk + (14 da.)	No liq	do	Neg.	Neg.	5.2	Below 55° C. (½ hr.).	(Not tested.)
1022	Antirabic vaccine.	Coccus .5μ	do	do	Orange.	Turbid++, no scum or ppt.	A ++ (2 da.) A+++ (14 da.)	Liq. 28 mm	+(24 hr.)	+	+	5.0	do	Pathogenic for rabbits and mice.
1025	do	Coccus .5μ	do	do	do	do	do	Liq. 31 mm	do	+	+	5.0	60° C. (½ hr.)	Pathogenic for rabbits in large amounts.
1031	Tuberculin	Coccus .8μ	do	do	White	do	No change (14 da.).	No liq	do	Neg.	Neg.	5.1	do	Nonpathogenic for rabbits and mice.
1041	do	Coccus .8μ	do	do	do	do	A+ (24 hr.) A+++(2 da.) curdled, reduced, partially peptonized (14 da.)	Liq. 27 mm	Neg	Neg.	Neg.	5.0	57½° C. (½ hr.)	Do.
1047	do	Coccus .8μ	do	do	do	do	A+(2 da.) curdled, reduced like B. Coli (7 da.).	Liq. 29 mm	do	Neg.	+	5.8	do	Do.
1049	Tetanus antitoxin.	Coccus .8μ	do	do	do	do	A+(14 da.)	Liq. 6 mm	do	Neg.	+	6.5	Below 55° C. (½ hr.).	Do.
1051	Antidysentery serum.	Coccus 1.0μ	do	do	do		No change (14 da.).	No liq	+?(24 hr.)	Neg.	Neg.	5.3	57½° C. (½ hr.)	Do.
1350a	Vaccine virus	Coccus .6μ	do	do	Orange.		A? partially peptonized. (24 hr.). Reduced ½ peptonized (14 da.).	Liq. 44 mm	+(24 hr.)	+	+	4.8	do	Pathogenic for rabbits and mice.

A = Acid. Alk = Alkaline.

DISCUSSION OF RESULTS.

Morphology.—All of the organisms of this group were cocci rranged in irregular groups, and varying in size from 0.5 to 1 μ n diameter. In general, these are larger than most of the forms onsidered by the Winslows in their classification of the *Coccaceæ.*

Gram stain.—By the method used for making the Gram stain all he organisms in this group were Gram positive. Several were shown y animal tests to be pathogenic (to be considered later), though the najority proved to be nonpathogenic. Winslow and Winslow[1] nd Kligler[2] consider positive Gram staining closely correlated with parasitic forms or with forms associated with life in or on the animal body, which would naturally include pathogenic forms and Gram negative forms as saprophytic. Churchman,[3] however, found saprophytic as well as parasitic forms Gram positive, using some of the same cultures as Kligler had tested. The difference appears to be a difference in interpretation of results rather than in methods of staining.

Pigment production.—Nos. 1022, 1025, and 1350a produced orange pigment. All others were white or colorless. All of the orange pigment producers were found to be pathogenic. Some of the white pigment producers were pathogenic and some nonpathogenic. None of the cultures produced a red or yellow pigment, yellow and red pigment production being considered by Winslow and Winslow as particularly characteristic of saprophytic forms.

Hemolysis.—The following cultures produced definite hemolysis in 24 hours: No. 1010, 1022, 1025, 1031, 1350a, and culture 1051 doubtful hemolysis. Of those showing definite hemolysis, all were pathogenic with the exception of cultures 1010 (tuberculin) and 1031 (tuberculin).

Reduction of nitrates.—Some of the forms which proved to be pathogenic, produced both nitrites and ammonia, which in the case of the cultures 1351a (vaccine virus) was very marked. Cultures 1022 (rabies vaccine) and 1025 (rabies vaccine) also showed positive tests for nitrites and ammonia.

Reduction of nitrates was not marked in any of the nonpathogenic cultures, though several showed a trace of nitrite. The production of ammonia was also not shown by any of these cultures with the exception of No. 1047 (tuberculin) and No. 1049 (tetanus antitoxin), the latter giving a good positive test on one occasion and a doubtful test on another.

The results obtained are not consistent throughout, but in general the results indicate that the pathogenic forms tend to reduce nitrates to nitrites and ammonia. The Winslows conclude from their investigation that the production of nitrites and especially ammonia is characteristic of Staphylococci isolated from the air.

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[1] *Loc. cit.*

[2] J. Infect. Dis., XII, 1913, p. 432.

[3] J. Exper. Med., XVII, 1913, p. 653.

H-ion concentration.—The results obtained with the dextrose-phosphate-peptone medium, while not proving entirely satisfactory, are nevertheless suggestive. In this study of *Coccaceæ* the pathogenic forms all reach a high H-ion concentration, while those which were found to be nonpathogenic showed considerable variation, some showing as high H-ion concentration as the pathogenic forms and some a much lower H-ion concentration.

All of the Staphylococcus cultures which were found to be pathogenic showed a pH value of about 5.[1]

	pH.
1022 (rabies vaccine)	5.0
1025 (rabies vaccine)	5.0
1350a (vaccine virus)	4.8
Staphylococcus (human) (control)	5.1
Staphylococcus (monkey) (control)	5.0

Two known pathogenic Staphylococci used as controls, the one isolated from abscesses in a monkey and the other from boils in man are seen to give the same results. (Staph. human and Staph. monkey.)

The nonpathogenic cultures show the following H-ion concentration:

	pH.
1010 (tuberculin)	5.0
1011 (tuberculin)	5.0
1014 (bacterial vaccine)	6.0
1016 (bacterial extract)	5.2
1031 (tuberculin)	5.1
1041 (tuberculin)	5.0
1047 (tuberculin)	5.8
1049 (tetanus antitoxin)	6.5
1051 (antidysentery serum)	5.1

Thermal death point.—The results of the tests for thermal death point do not show that the nonpathogenic forms among these cultures were necessarily more resistant to heat than the pathogenic, several of the nonpathogenic forms being killed at 55° C., while several pathogenic forms resisted temperatures up to 60° C. for ½ hour.

Pathogenicity.—The pathogenicity of the various cultures was tested on rabbits and mice, the rabbits being inoculated both subcutaneously and intravenously. In a few cases tests were made on guinea pigs and rats. In all cases 24 hr. old broth cultures were used for inoculations.

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[1] P^{H} is the empirical symbol used to express the logarithm of the reciprocal of the H-ion concentration, i. e., the reaction of the medium produced as a result of the breaking down of the particular carbohydrate used by the action of the organism. That this value is fairly constant under certain conditions and therefore useful as a means of identification of certain organisms has recently been shown by Clark and Lubs (J. Infec. Dis., vol. 17, 1915, p. 160–173) and others.

The following summarizes the results of animal tests for those organisms which proved to be pathogenic:

No. 1022, from antirabic vaccine, sometimes produced a remarkable board-like hardening at the site of inoculation in rabbits and guinea pigs on subcutaneous injection and caused the death of a rabbit injected intravenously with 0.1 c. c. in 6 days, giving rise to abscesses in the internal organs from which the organism was recovered.

No. 1025, also from antirabic vaccine, gave rise to abscesses at the site of inoculation in mice.

One organism from vaccine virus (1350a) was irregularly pathogenic for rabbits and mice, causing death in 1 to 12 days, but gave no characteristic lesions.

Summary of results with Coccaceæ (cells in irregular groups).—Reviewing the cultural characteristics of the organisms which were pathogenic, we find them to be all typical Staphylococci, Gram positive, growing well on agar, with the production of orange pigment, producing turbidity in broth with no scum, acidifying litmus milk, with curdling and peptonization in the case of No. 1350a (vaccine virus), liquefying gelatin, producing hemolysis on blood agar, reducing nitrates, reaching a pH value of about 5.0 in dextrose-phosphate-peptone medium, and resisting temperatures varying from below 55° to 60° C. for ½ hour.

Greisse,[1] in his work on the differentiation of pathogenic from saprophytic Staphylococci, found hemolysis on blood agar never absent with pathogenic staphylococci, but that the majority of non-pathogenic forms also produced hemolysis, though more slowly and less strongly; that gelatin was rapidly liquefied by pathogenic and more slowly by saprophytic strains, litmus milk was little changed by pathogenic forms, but strongly acted upon by saprophytic forms; that white forms predominated among the saprophytic forms, 42 per cent of the *aureus* strains tested by him being found to be pathogenic, while only 4.8 per cent of the white forms were pathogenic.

The results obtained with the cultures from biological products tested as to pathogenicity agree in general with the above, with the exception of the behavior in litmus milk, it being found that the non-pathogenic forms were not more active as a rule than the pathogenic.

Following the classification of Winslow and Winslow, cultures 1022 (antirabic vaccine), 1025 (antirabic vaccine), and 1350a (vaccine virus), which produced an orange pigment, with cells in groups, which were Gram positive, grew well on agar, liquefied gelatin, and produced acid in dextrose medium, would be classified in the genus *Aurococcus*, and since they reduced nitrates in the species *Aur.*

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[1] Ztschr. f. Hyg. u. Infektionskrankh., Lpz., 1913, LXXVI, 282–306.

mollis. This organism is described as "a parasitic coccus, living normally on the surface of the human or animal body, or in diseased tissues; often found in the air." Considering the nature of the products from which these organisms were isolated, rabies vaccine and vaccine virus, and the fact that they were pathogenic, it is probable that they were derived from animal sources. The method of preparation of these products does not exclude the presence of Staphylococci in association with the product, though the use of glycerin in their preparation might be expected to destroy these organisms.

The remaining *Coccaceæ*, 1010 (tuberculin), 1011 (tuberculin), 1014 (bacterial vaccine), 1016 (bacterial extract), 1031 (tuberculin), 1041 (tuberculin), 1047 (tuberculin), 1049 (tetanus antitoxin), 1051 (antidysentery serum), produced white pigment. Winslow and Winslow consider separately the saprophytic cocci (yellow and white forms) and the parasitic cocci.

The white saprophytic forms are described as being generally Gram negative, and nonacid formers, and only a nonliquefying organism is described. Of the above cultures, none, with the possible exception of No. 1014 (bacterial vaccine) and No. 1049 (tetanus antitoxin), which had comparatively low H–ion concentration and liquefied gelatin only slightly after being retained 30 days, correspond to this description (*Micrococcus candicans*).

The remaining cultures, all Gram positive, and producing acid in dextrose (pH 6.0 to 5.0), answer Winslow and Winslow's description of the genus *Albococcus*. Nos. 1010 (tuberculin), 1011 (tuberculin), 1016 (bacterial extract), 1031 (tuberculin) and 1051 (antidysentery serum), did not liquefy gelatin and therefore correspond to the species *Alb. candidus* under this classification. Nos. 1041 (tuberculin) and 1047 (tuberculin) liquefied gelatin, but did not reduce nitrates, classifying, therefore, with *Alb. pyogenes.*[1]

Out of the 500 strains of cocci studied by the Winslows, 23 were classified as Albococci, and some were derived from the body and some from the air.

It is probable that some of the above cultures from biological products (white Staphylococci) were derived from the air, though contamination may also have occurred through the handling of apparatus and materials used in the preparation of the products.

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[1] The more recent classification recommended by the committee of the Society of American Bacteriologists on characterization and classification of bacterial types (J. Bact., ii, 1917, 505), places orange pigment producing forms under *Staphylococcus*, of which the type species is *Staphylococcus aureus* (Nos. 1022, 1025, 1350a). Cocci producing white pigment and which liquefy gelatin less vigorously than the preceding are classified under *Albococcus* (Nos. 1010, 1011, 1014, 1016, 1031, 1041, 1047, 1049, 1051). The *Micrococcaceæ*, distinguished by Gram negative staining and production of red or yellow pigment, are not represented by any of the cocci studied.

It is to be admitted, however, that it is not possible to determine with any degree of certainty the origin of any particular organism when a limited number of organisms which appear to be similar are derived from a number of different products which are prepared by different methods and under different conditions.

(b) *Cells in chains—Methods.*—In general, the same methods were employed in the study of this group as in the preceding, to which reference may be made.

TABLE II.

No.	Products from which isolated	Morphology.	Gram stain.	Agar.	Pigment.	Litmus milk.	Gelatin. Liquefaction (30 da.).	Hemolysis.	Reduction of nitrates.		H-ion concentration.	Thermal death point.	Pathogenicity.
									Nitrites.	NH_3.			
1006	Diphtheria antitoxin.	0.8 μ (10–12 elements).	Positive..	Thin, transparent growth.	Colorless.	Acid (14 da.)..	Neg.......	Neg..	Neg...	Neg..	p*H*. 5.0	60° C. (½ hr.)..	Nonpathogenic for rabbits and mice.
1007	...do.............	.....do........	...do.....	.....do......	...do.....	.....do........	...do......	Neg..	Neg...	Neg..	4.8	.....do.........	Not tested.
1021	Vaccine virus.......	0.5 μ (10 elements).	...do.....	.....do......	...do.....	.....do........	...do......	+....	Neg...	Neg..	5.2	Below 55° C. (½ hr.).	Pathogenic for rabbits and mice.
1043	Diphtheria antitoxin	0.7 μ (5–6 elements)	...do.....	.....do......	...do.....	.....do........	...do......	Neg..	Neg...	Neg..	4.8	62½° C. (½ hr.).	Nonpathogenic for rabbits and mice
1413	Bacterial vaccine (autogenous).	0.6 μ (7–8 elements).	Negative.	.....do......	...do.....	Acid, curdled (14 da.).	...do......	Neg..	Neg...	Neg..	4.8	Below 55° C. (½ hr.).	Do

DISCUSSION OF RESULTS.

Growth on agar.—All of the above cultures, after cultivation in agar stabs for some time, grew well on the surface of slant agar, the characteristic transparent film being produced.

H-ion concentration.—The following results were obtained in testing H-ion concentration in the special dextrose medium used as described under *Coccacea* (cells in groups) and in lactose medium.

	Dextrose.	Lactose.
	pH.	pH.
1006 (diphtheria antitoxin)	5.0	5.2
1007 (diphtheria antitoxin)	4.8	5.6
1021 (vaccine virus)	5.2	5.8
1043 (diphtheria antitoxin)	4.8	5.4
1413 (bacterial vaccine, autogenous)	4.8	....
K (control culture, ear abscess)	6.0	....

Ayers[1] found that Streptococci derived from human infections reached a lower H-ion concentration than those derived from other sources, the former as a rule giving a pH value about 5.5 to 6.0, and the latter 4.6 to 4.8. The result obtained with a control culture K derived from an ear abscess confirms this result. Culture 21 (vaccine virus) which was the only one of this group which proved to be pathogenic for rabbits and mice showed a H-ion concentration in dextrose only slightly lower than the other nonpathogenic cultures. The results obtained in the case of the latter confirm Ayers's conclusion, that forms nonpathogenic for man reach a relatively high H-ion concentration. However, there were not enough cultures tested in this group to draw any very definite conclusions.

Thermal death point.—Cultures 1021 (vaccine virus) and 1413 (autogenous vaccine) were killed by a temperature of 55° C. maintained for one-half hour. Cultures 1006 and 1007 (diphtheria antitoxin) grew after heating at 57½° for one-half hour, but were killed by 60° for the same length of time. Culture 1043 (diphtheria antitoxin) was most resistant, surviving a temperature of 60° for one-half hour and being killed at 62½°. Culture 1021, the only one pathogenic for rabbits and mice, was therefore killed by a lower temperature than the others (except 413).

Pathogenicity.—But one of these cultures No. 1021 (vaccine virus) proved pathogenic for mice and rabbits, 0.5 c. c. of a broth culture killing a mouse in 3 days, and 2 c. c. injected intravenously by killing a rabbit in 10 days. The lesions were not characteristic. In one case the organism was recovered from the heart's blood of a mouse *post mortem.*

It is not possible to determine definitely the origin of the Streptococci isolated. The only one, No. 1021, from vaccine virus, which was found to be pathogenic for animals was also the only one showing hemolysis on blood agar. Probably this organism was present as contamination of the virus as taken from the animal, which might be expected from the nature of the preparation of vaccine virus.

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[1] Journ. Bact., I, 1916, 84–85.

The other Streptococci were probably saprophytic forms. Nos. 1006 and 1007, and 1043, all from diphtheria antitoxin, were not pathogenic for rabbits or mice, and did not produce hemolysis on blood agar. Some acid was produced in lactose broth (pH 5.6 to 5.2). Nonfermentation of lactose has been considered by some authors as characteristic of equine streptococcus strains, though there is a lack of agreement on this point among various authors. Broadhurst[1] in "An environmental study of Streptococci with special reference to fermentative reactions" found that 61 per cent of 44 equine strains tested, fermented lactose, and also found failure to cause hemolysis characteristic of strains from equine feces. As a result of an extensive study this author decides that fermentation tests are not definitely helpful in determining the origin of a given streptococcus.

The member of Streptococci isolated from diphtheria antitoxin in this study are too few to indicate anything conclusive in regard to source, but it seems probable that they were of equine origin.

Culture No. 1413 isolated from a bacterial vaccine (autogenous), was a Gram negative organism, was nonpathogenic for animals and did not produce hemolysis, and appears to be a saprophytic form.

2. NONSPORE-BEARING RODS.

In the study of this group, the methods used in general under *Coccaceæ* were employed, but since there were a number of different species, most of which were represented by only one organism, the results are not presented in tabular form, but each species is discussed separately.

Cultures Nos. 1040, 1046, 1256, and 1268, all from tetanus antitoxin, and 1059 (antimeningitis serum), will be considered first.

Morphology and Gram stain.—Morphologically these cultures appeared to be the same organism. All were small rather thick rods, measuring about .5 by 1.5μ, and showing a characteristic parallel arrangement, usually in pairs, but sometimes in groups of 4 or 5. They were all Gram positive, the same method of staining being employed as under the cocci. All of these cultures showed slight motility.

Spores could not be demonstrated in any of these cultures, though old cultures kept at a low temperature (15° C.) for several months and cultures kept for 30 days at incubation temperature were examined.

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[1] Jour. Infect. Dist., XVII, 1915, 277.

Agar.—The organism produces a rather thick elevated growth on agar which in older cultures becomes dry and crumbly.

Broth.—The above cultures all produced a slight amount of turbidity in broth, but no scum production. The growth in dextose broth was particularly characteristic of this organism, a fringy growth extending downward from the surface of the broth and clinging to the sides of the tube being produced. This appearance was not noticeable in the growth of cultures when first isolated, but was very marked after the organism had been under cultivation for some time.

Litmus milk.—No change was apparent in litmus milk in 48 hours. At the end of 14 days all showed a slightly alkaline reaction.

Gelatin.—None of this group liquified gelatin in 30 days.

Reduction of nitrates.—Reduction of nitrates was tested for by the same method as employed under cocci. All reduced nitrates to nitrites with the exception of No. 1046 (tetanus antitoxin) and none produced ammonia.

H-ion concentration.—All showed a H-ion concentration of 6.7–7.0 in the special dextrose medium, though growth was very scant.

Thermal death point.—The following numbers were killed by a temperature of 55° C. for one-half hour: 1059 (antimeningitis serum), 1256 (tetanus antitoxin). No. 1268 (tetanus antitoxin) was killed by a temperature of 57.5°, while culture 1046 (tetanus antitoxin) survived a temperature of 60°, but was killed by a temperature of 62.5°.

Pathogenicity.—Tests were made on mice and rabbits with 24-hour old broth cultures. One c. c. injected intravenously killed rabbits in 2 to 21 days. Some of the mice injected with 1 c. c. died in 4 to 6 days. The organism was not recovered *post mortem.*

The organisms isolated from the four different specimens of products, tetanus antitoxin (Oct., 1915), tetanus antitoxin (Oct. 25, 1916), tetanus antitoxin (Oct. 20, 1916), and antimeningitis serum, appear to be identical morphologically, and culturally, the only variation being in the thermal death point. The organism is somewhat variable in its behavior, after having been under cultivation for some time. Growth on agar became more crumbly and the fringed appearance in dextrose broth more marked.

Occurring as the organism does in the same class of products, in tetanus antitoxin three times, and in antimeningitis serum once, the indication is that it may be associated with horses or the stables in which the horses used in the preparation of the products are kept. The fact that it is somewhat pathogenic for rabbits and mice also indicates that it may be derived from animal sources.

Culture No. 1015 (antidysentery serum) appeared to be a distinct organism, characterized by the production of an orange pigment. The organism was a Gram positive rod and slightly motile, and grew well on agar. No change was produced in the reaction of litmus milk in 14 days, the growth appearing as an orange precipitate in the bottom of the tube. A slight turbidity was produced in broth. Gelatin was not liquefield in 30 days. Nitrates were reduced to nitrites, but no ammonia was found. The organism was killed by heating to a temperature of 55° C. for half an hour.

A pH value of 6.7 was produced in the special dextrose medium. 1 c. c. of a 24-hour broth culture injected intravenously into a rabbit produced no effect. A mouse inoculated with the same amount died in 6 days, but the organism was not recovered.

This organism corresponds closely to the description of *B. aurantiacus* isolated from water described by G. and P. Frankland[1] and is probably a saprophytic form.

Culture No. 1353 (vaccine virus) isolated from the same vaccine virus from which pathogenic Staphylococci were isolated was a Gram negative small rod, measuring .5 by .7 to .8 μ. The organism grew well on agar, produced acid in litmus milk in 24 hours and curdled and partially peptonized the casein in 14 days. Gelatin was liquefied in 30 days. The organism reduced nitrates to nitrites and ammonia, and was killed by a temperature of 55° C. for one-half hour.

This organism seemed to be somewhat pathogenic, a rabbit succumbing after intravenous inoculation of a large dose and a mouse after subcutaneous inoculation.

Cultures No. 1008 (tetanus antitoxin) 1012, and 1013 (bacterial extract), 1269 (tetanus antitoxin), 1320 (brain extract), and 1352 (vaccine virus) were all very short rods resembling cocci.

Culture No. 1008 isolated from tetanus antitoxin was distinguished by its production of a bright yellow pigment. The organism a small rod, about .5μ long. Growth on agar was luxuriant. The organism was Gram positive and slightly motile. Turbidity was produced in broth, with no scum or precipitate. No change was produced in litmus milk in 14 days. Gelatin was liquefied to a depth of 27 mm. in 30 days. The thermal death point of this organism was below 55° after heating one-half hour, showing it to be less resistant to heat than the majority of the organisms tested. Nitrates were not reduced to nitrites, but ammonia was formed. The H-ion concentration in the special dextrose medium was 6.4. No effect was produced by intravenous or subcutaneous inoculations in rabbits or subcutaneous inoculations in mice.

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[1] Ztsch. f. Hyg., vol 6, 1889, p. 390.

Cultures No. 1012 and 1013 from a bacterial extract were very small coccoid-bacilli about .4µ in length. Growth on agar was scant, a thin translucent film, resembling Streptococcus growth, being formed. The organism was Gram positive and slightly motile. Broth was rendered turbid, with no scum production, and litmus milk was acidified and rapidly curdled and peptonized. Action in gelatin was very vigorous, the whole tube being rapidly liquefied. A trace of nitrites but no ammonia was formed from nitrates. A H-ion concentration of 4.8 was produced in the dextrose medium. This organism was more resistant to heat than the majority of organisms tested for thermal death point, a temperature of 62° C. for one-half hour being required to kill. A rabbit inoculated intravenously with 1 c. c. of a broth culture of this organism was killed in 14 days, but no effect was produced by subcutaneous inoculation in rabbits, guinea pigs, rat, or mouse, except a hardening at the site of inoculation in the case of the rabbit.

Culture No. 1269 isolated from tetanus antitoxin was a large coccoid bacillus about 1.2µ in length, often occurring in pairs and tetrads. The organism was Gram positive, nonmotile and grew well on agar, but was very inactive in all other culture media in which it was tested, no change being produced in litums milk except a very slight alkalinity, in 14 days, no liquefaction of gelatin taking place in 30 days and nitrates not being reduced. The thermal death point was below 55° for one-half hour. A H-ion concentration of 6.7, or practically no acid, was produced in the dextrose medium. A large amount, 2 c. c. of a broth culture, injected intravenously killed a rabbit in 13 days, but smaller doses had no effect on either rabbits or mice.

Culture No. 1320 isolated from brain extract was a small coccobacillus about .6µ long and often occurring in chains. The organism was Gram positive and nonmotile. Growth on agar was not very vigorous. This organism was also inactive in other media, no change being produced in litmus milk or gelatin, and nitrates were not reduced. A high H-ion concentration, however, 4.6, was produced in the dextrose medium. This organism was distinguished by a higher thermal death point than any of the other cultures tested, a temperature of 65° C. for one-half hour being required to destroy. No effect was produced by inoculating rabbits and mice with broth cultures.

Culture No. 1352 from vaccine virus was a gram negative coccoid-bacillus about .6µ long. Growth on agar was good, an alkaline reaction was produced in litmus milk, no liquefaction of gelatin was produced and nitrates were not reduced. A H-ion concentration of 5.8 was produced in the dextrose medium. The thermal death point was 57½° C. for half an hour. This organism was pathogenic for rabbits and mice, a rabbit receiving 1 c. c. broth culture intravenously being killed in 2 da. and a mouse. 1 c. c. broth culture subcutaneously in 1 day.

The above cultures of coccoid bacilli (Nos. 1008, 1012, 1013, 1269, 1320, and 1352) could not be definitely identified with any of the organisms described in the literature as far as examined.

All of the non-spore bearing rods (including coccoid forms) studied, were derived from serums, antitoxins and vaccine virus, with the exception of Nos. 1012-1013 (from bacterial extract). Since some of these were found to be pathogenic for laboratory animals, it is probable that they were derived from animal sources. The pigment producing forms, No. 1008 (tetanus antitoxin) and No. 1015 (antidysentery serum) appear to be saprophytic forms.

3. SPORE-BEARING RODS.

All of the spore-bearers studied were aerobic with the exception of culture 1039 (bacterial vaccine), which grew only under anaerobic conditions.

Reference was made to the work of Ford[1] on Aerobic spore-bearing non-pathogenic bacteria as well as to Chester,[2] Gottheil,[3] Migula,[4] and Sternberg[5] in the study of the aerobic spore-bearers. The earlier descriptions of spore-bearing organims are indefinite and seem to be of little value in the identification of the various spore-bearing species. The work of Ford and collaborators who examined over 1,700 cultures from various sources, milk, soil, dust, water, intestinal contents and contaminated plates, was found to be more satisfactory.

In the beginning of the work the majority of the tests used by Ford were employed, including morphology, motility, staining properties, spore formation, growth on agar slant, agar stab, agar colonies, litmus glucose agar slant, gelatin stab, broth, peptone, potato, litmus milk, blood serum, dextrose saccharose and lactose fermentation. Later several of these tests were omitted, viz, agar stab, litmus dextrose agar, peptone, potato, blood serum and the place of the fermentation tests of dextrose, lactose and saccharose determinations of H-ion concentration in special dextrose medium (1 per cent peptone, 1 per cent dextrose, 1 per cent dibasic potassium phosphate) were made.

Morphology, the nature of the growth on slant agar, and scum formation on broth were useful for classifying the organism broadly by groups. Those showing a dry penetrating growth on agar, with tenacious scums on fluid media are classified as *Subtilis* or *Mycoides* in Ford's arrangement. Those which grow as a soft mass on agar and which are easily scraped from the surface of the agar are classified as *Mesentericus* or *Cereus*. Very large organisms, which grow as a thick mass on agar and which produce no scum in broth belong to the *Megatherium* group. In addition to these groups are the *Cohaerens-simplex* group, a smaller organism than *Megatherium*, which grows as a soft mass on agar and produces no scum in broth, the round terminal spored group easily distinguished by morphology, the central spored group, with spindle-shaped organisms, and the cylindrical terminal spored group.

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[1] Jour. Bact. I, 1916, 273–318, 493–533.

[2] 15th Ann. Rept. Delaware College Agr. Exper. Sta. 1903.

[3] Centralbl. f. Bakt., 2 Abt. VII, 430, 1901.

[4] Migula, W. System der Bakterien, 1897–1900.

[5] Sternberg, G. M. A manual of bacteriology, 1893.

Representatives of nearly all of the groups were found among the cultures studied.

The group represented by the largest number of organisms was found to be the *Mesentericus* group. The organism described by Ford as B. *subtilis* was found only three times, though there is considerable confusion in the identification of this organism in the literature, some authors considering it identical with B. *mesentericus vulgatus*.

The cultures classified by groups follow:

B. subtilis group. –No. 1018 (bacterial vaccine), No. 1225 (bacterial vaccine), No. 1415 (bacterial vaccine).

The organisms belonging to this group were rather thick rods varying in size from .5 to .6 by 2 to 4.0μ. Spores located centrally were present at the end of 24 hours' growth. All were Gram positive and motile. The growth on agar was especially distinctive, being dry and penetrating and could be removed from the agar with difficulty. The organism was strictly aerobic, and a flaky scum appeared on the surface of broth within 24 hours and this later developed into a firm tenacious scum. No change was apparent in litmus milk in the first day or two, but it was gradually peptonized to an amber colored fluid. Gelatin was rather rapidly liquefied with scum formation. Colonies on agar appeared as a rather firm scum enclosing a gelatinous fluid in the case of Nos. 1225, 1415. No. 1018 showed dry irregular colonies which could be broken up with difficulty. H-ion concentration determinations were made, using the same medium as in the study of the *Coccaceæ*, with the following results: No. 1018, 5.6; No. 1225, 5.8; No. 1415, 5.6.

Mesentericus group—*B. Mesentericus.*—No. 1001 (bacterial vaccine), No. 1002 (bacterial vaccine), No. 1044 (bacterial vaccine), No. 1097 (bacterial vaccine), No. 1240 (bacterial vaccine), No. 1244 (bacterial vaccine), No. 1188 (bacterial vaccine), No. 1196 (thyroidectomized horse serum), No. 1414 (bacterial vaccine).

All of the organisms of this group were small rods varying in size from 0.5 to 0.6 by 1.8 to 4µ. Some showed a tendency to chain formation. Spores were small and oval and usually appeared within 24 hours. All of these organisms were Gram positive and motile, though some were more actively motile than others. Growth on agar was moist and luxuriant with a tendency to wrinkle in several of the cultures. Broth was rendered turbid in 24 hours and scum was present in 48 hours in all of the cultures except No. 1240 and 1414. Litmus milk, as in the case of *B. subtilis*, showed no change in one or two days, after which peptonization took place slowly. Gelatin was liquefied by all these cultures, with scum production. On agar plates, round raised, moist colonies up to about 1 mm. in diameter were formed.

The following pH values were obtained in the special dextrose medium:

	pH.
No. 1001	5.2
No. 1002	5.5
No. 1044	5.6
No. 1097	5.2
No. 1240	5.3
No. 1244	5.4
No. 1188	5.9
No. 1196	5.9
No. 1414	5.4

B. Globigii.—No. 1204 (bacterial vaccine); No. 1440 (bacterial vaccine.)

These two organisms were rods of from .5 to .6µ in thickness and 1.8 to 5.5µ in length. In the case of culture No. 1204 no spores were visible in 24 hours, but were present in 7 days. No. 1440 showed swollen forms in 6 hours and spores in 24 hours. The spores were thicker than the rods in both cases.

Culture 1204 was Gram negative and 1440 doubtfully Gram positive. Both showed active motility. Growth on agar was scant in both. Broth was rendered slightly turbid, and neither scum nor precipitate formed. Litmus milk was reduced in 24 hours and became acid in two days, and at the end of 14 days was markedly acid with no curdling or peptonization. Liquefaction of gelatin was not present in 7 days, but both showed some liquefaction in 30 days. Small round colonies were produced on agar plates.

pH values in the special dextrose-phosphate-peptone medium were as follows: No. 1204, 5.8; No. 1440, 5.4.

B. Megatherium group.—Only one representative of this group was found, No. 1190 (bacterial vaccine).

This organism was a very large rod, with rounded ends, 1.2 by 3.5 to 5.4μ, with some very long filaments present. No spores were visible in 24 hours, but they were numerous in 7 days. The organism was Gram positive and slightly motile. Growth on agar was thick, raised, and creamy and showed pellucid areas. Broth became turbid in 24 hours, and no scum was found in 14 days. Peptonization of litmus milk began within 24 hours, and was complete in 7 days, with the formation of an amber colored fluid and flocculent precipitate. Gelatin was rapidly liquefied with no scum production. Colonies on agar were small and round and somewhat wrinkled. A pH value of 6.5 was obtained in the special dextrose medium, though growth was very scant.

Cohaerens-simplex group.—No. 1201 (bacterial vaccine); No. 1297 (bacterial vaccine).

These two organisms while not corresponding exactly to either of the two cultures *B. cohaerens* or *B. simplex* as described by Ford have been placed here, as they appear to belong more properly in this group than any of the others. Spores were formed more rapidly in the two members of this group than described by Ford, being present in 24 hours, and were wider than the vegetative forms. Growth on agar was fairly luxuriant, while the organism *B. cohaerens* and *B. simplex* are described as showing a thin growth on agar. Also no change was produced in litmus milk by the cultures studied, whereas in the case of the two above organisms (*B. cohaerens* and *B. simplex*) peptonization of milk is described by Ford.

The two cultures showed thick rods .6 by 1.5 to 2.5μ. Both were motile and Gram positive. Broth became turbid in 24 hours, and no scum or precipitate was formed. Gelatin showed liquefaction in 24 hours and at the end of thirty days both tubes were liquefied to a depth of 19-20 mm. Agar plates showed small, round white colonies.

Both cultures failed to grow in the special dextrose medium used for H-ion concentration determinations.

B. Cereus.—No. 1004 (tuberculin); No. 1005 (tuberculin); No. 1033 (tetanus antitoxin); No. 1042 (tuberculin); No. 1176 (bacterial vaccine); No. 1361 (horse serum); No. 1421 (bacterial vaccine); No. 1422 (bacterial vaccine).

These organisms were all large rods, measuring 0.8 to 1.5 by 2 to 5.5μ, with a tendency to grow in chains. Spores were present in 24 hours or less in all of the cultures, usually centrally located and slightly wider than the rods. All were Gram positive and showed more or less marked motility.

Growth on agar was very luxuriant, with a tendency to wrinkle, and was easily removable from the surface. Broth was clear or slightly turbid in 24 hours and a heavy wrinkled scum was found within two days, the medium being perfectly clear under the scum in cultures 1033, 1176, 1361, 1421 in 7 days. Later the medium again became turbid in 1361, 1421, and 1422.

Gelatin was acted upon vigorously, showing some liquefaction in 24 hours and a large amount in 3 days. A scum was produced by all these cultures.

Litmus milk was rapidly acted upon by most of the cultures, all except 1033 and 1421 showing some curdling and peptonization in 24 hours. All were practically completely peptonized in 14 days.

Colonies on agar were dry with irregular edges, and the whole colony could easily be lifted from the agar in the case of 1033, 1361, 1421, and 1422.

pH values in the special dextrose medium were as follows: No. 1004, 5.1; 1005, 5.0; 1033, 6.5; 1042, 5.2; 1176, 5.2; 1361, 6.6; 1421, 6.4; 1422, 6.2.

The two ranges in H-ion concentration and differences in appearance of agar colonies and behavior in broth indicate two types in this group.

Round terminal spored group—B. Pseudotetanicus.—No. 1009 (tetanus antitoxin); No. 1032 antipneumococcic serum); No. 1035 (antirabic vaccine); No. 1037 (antirabic vaccine).

The organisms in this group were quite distinctive, being Gram negative and showing very early spore formation (6 hours), the spores being terminal or subterminal and wider than the organism, resembling the tetanus organism. The vegetative cells measured .6 by 1.8 to 6μ. Spores were oval and abut 1 by 1.8μ. All of the cultures showed some motility. Growth on agar slant was not as luxuriant as was that of the majority of spore bearers. Broth became only slightly turbid in 14 days, and no scum was found. Gelatin was not liquefied by any of the cultures in 30 days. Litmus milk showed no change in two days, and at the end of 14 days showed a very marked alkaline reaction, or the color was completely reduced. The following pH values were obtained in the dextrose medium: 1009, 6.4; 1032, 6.5; 1035, 6.6; 1037, 6.6.

Cylindrical terminal spored group—B. circulans Jordan.—Two organisms were classified in this group: No. 17 (bacterial vaccine), No. 19 (bacterial vaccine).

These two organisms appeared similar in most respects, though No. 1017 was uniformly Gram negative and No. 1019 was apparently Gram positive. Both were in the same product, though in containers having different laboratory numbers. The vegetative cells were thin bacilli, measuring 0.6 to 0.9 by 2 to 9μ. The spores were wider than the rods, and terminal or subterminal. Both were slightly motile. Broth was rendered slightly turbid and no scum or precipitate was found. Litmus milk became acid and was curdled by culture 1019. Neither culture produced liquefaction of gelatin in 30 days. Colonies on agar were small and round, but in three or four days larger colonies up to 3 mm. diameter were present. Both showed a pH value of 6.6 in the special medium used, though growth was scant.

The spore-bearing organisms found as contaminations in biological products are probably present for the most part as the result of accidental contamination with organisms from the air in the process of manufacture and filling. Only one form, that classified as *B. pseudotetanicus*, appears to have been associated exclusively with the class of products derived from animal sources. This organism was present in antirabic vaccine, tetanus antitoxin, and antipneumococcic serum. The remainder of the spore-bearing organisms, including representatives of nearly all of the groups described by Ford, were for the most part present in the bacterial vaccines. A horse serum, which contained several varieties of spore-bearing organisms, was an exception.

Anaerobic spore bearers.—The only anaerobic culture was No. 1039, isolated from a bacterial vaccine. This organism appeared morphologically and culturally somewhat similar to tetanus, but did not cause tetanus symptoms in animals.

The organism was a Gram positive rod. No spores were visible in 24 hours, but in 48 hours a few swollen rods were present and in 4 days rounded terminal spores were present. The organism was motile.

The organism grew well in the depths of agar and produced an odor resembling that of the tetanus organism. A slight amount of gas was produced in dextrose, lactose, and saccharose fermentation tubes. Gelatin was liquefied.

A mouse, guinea pig, and rabbit were each given 1 c. c. of a broth culture subcutaneously, but remained free from symptoms.

SUMMARY.

A study of organisms isolated as contaminations in biological products during the period from November, 1914, to January, 1917, include representatives of the following groups: *Coccaceæ* (Staphylococci, Streptococci), spore-bearing rods (aerobic), spore-bearing rods (anaerobic) and nonspore-bearing rods, including coccoid bacilli. Aerobic spore-bearing rods were most often isolated, though Staphylococci were also frequently encountered.

To a certain extent the nature of the contamination appears to bear some relation to the kind or class of products. Characteristic nonspore-bearing rods, identical morphologically and culturally, were isolated three times from tetanus antitoxin and once from antimeningitis serum. An aerobic spore bearer classified as *B. pseudotetanicus* was isolated twice from rabies vaccine, once from tetanus antitoxin, and once from antipneumococcic serum. *Staphylococcus aureus* was isolated twice from rabies vaccine and from vaccine virus. Streptococci were present twice in diphtheria antitoxin and once in vaccine virus. The bacterial vaccines and tuberculins were contaminated for the most part with spore-bearing organisms and nonpathogenic staphylococci. Coccoid bacilli of several different species were present in tetanus antitoxin, brain extract, and vaccine virus.

Organisms which proved to be pathogenic for rabbits and mice were isolated from vaccine virus, rabies vaccine, tetanus antitoxin, and one bacterial product. These included *Aurococcus*, *Albococcus*, two nonspore-bearing bacilli, and two species of coccoid bacilli. No pathogenic organisms were isolated from any of the bacterial vaccines with one exception, that of a bacterial extract, from which an organism which was somewhat pathogenic was isolated. In general, however, it appears that contaminations of the bacterial vaccines, tuberculins, and other products similarly prepared are saprophytic forms.

Staphylococci, streptococci, and certain nonspore-bearing rods and coccoid bacilli, derived from the class of products, including serums, and antitoxins are probably frequently derived from animal sources and may be pathogenic. It would seem desirable when such forms are encountered in the testing of biological products of the above classes for sterility, that the organism be isolated and tested further for pathogenicity and cultural reactions.

STUDIES IN PRESERVATIVES OF BIOLOGICAL PRODUCTS—THE EFFECTS OF CERTAIN SUBSTANCES ON ORGANISMS FOUND IN BIOLOGICAL PRODUCTS.

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It is customary to add a small amount of an antiseptic to serums, bacterial vaccines, and other biological products to prevent the multiplication of organisms which may get into them at some stage of manufacture or use; usually a member of the phenol group is used in proportions not to exceed 0.5 per cent. Chloroform and glycerin are used for special purposes. Experience at this laboratory and at manufacturing establishments has shown that, in spite of the presence of preservatives, contaminations are occasionally found in finished products.

In this work certain of these contaminating organisms have been used as test objects for the antiseptic action of some of the substances used, or suggested for use, as preservatives of biological products. Only ærobic nonspore-bearing organisms have been considered. These cultures will be referred to by number and a brief description of their characteristics is given (Table 1).

Table I.

The cultures from biological products used were as follows:

Culture No.	Morphology.	Reaction to Gram's stain.	Pigment production.	Pathogenicity.
6	Streptococcus, diam. 0.8 micron, chains 10–12 elements.	+	None	1 c. c. intravenously in rabbit; no effect. 1 c. c. subcutaneously in mouse; no effect.
8	Coccus, diam. 0.5 micron, occurs in pairs and short chains.	+	Yellow	2½ c. c. broth culture subcutaneously in rabbit; no effect. 1 c. c. intravenously in rabbit; no effect. 1 c. c. subcutaneously in mouse; no effect.
10	Staphylococcus, diam. 0.8 micron.	+	White	2½ c. c. broth culture subcutaneously in rabbit; no effect. 1 c. c. intravenously in rabbit; no effect. 1 c. c. subcutaneously in mouse; no effect.
11	Coccus, diam. 0.7 micron, occurs in pairs and tetrads.	+	do	1 c. c. broth culture intravenously in rabbit; dead in 12 days, shows pyothorax and abscess in connective tissue of neck. 1 c. c. broth culture subcutaneously in mouse; dead in 4 days; shows abscess of liver.
12	Cocco-bacillus, diam. 0.4 micron.	+	None	2½ c. c. subcutaneously in rabbit; no effect except induration at site of injection. 1 c. c. intravenously in rabbit; dead in 3 days; no lesions. Mouse, rat, guinea pig 1 c. c. subcutaneously; no effect.
13	do	+	do	See No. 12.

Culture No.	Morphology.	Reaction to Gram's stain.	Pigment production.	Pathogenicity.
14	Staphylococcus, diam. 0.8 micron.	+	White	1 c. c. broth culture intravenously in rabbit; no effect. 1 c. c. broth culture subcutaneously in mouse; no effect.
16	do.	+	None	Not tested.
22	Staphylococcus, diam. 0.5 micron.	+	Orange	$\frac{1}{10}$ c. c. broth culture intravenously in rabbit produced a general pyemia. 1 c.c. broth culture subcutaneously in rabbit; dead in 6 days; board-like infiltration of subcutaneous tissue of abdomen. 1 c. c. broth culture in mouse; dead in 1 day.
25	do.	+	do.	2 c. c. broth culture intravenously in rabbit; dead in 1 day. ½ c. c. as above; no effect. 1 c. c. subcutaneously in mouse; abscess at site of inoculation. Did not kill in 30 days.
31	Staphylococcus, diam. 0.8 micron.	+	White	2 c. c. broth culture intravenously in rabbit; no effect. 1 c. c. subcutaneously in mouse; no effect.
40	Rods, 0.5 by 1.2 micra.	+	do.	1 c. c. broth culture intravenously in rabbit; dead in 21 days. 1 c. c. broth culture subcutaneously in mouse; dead in 6 days.
41	Staphylococcus, diam. 0.8 micron.	+	do.	2½ c. c. broth culture subcutaneously in rabbit; no effect. 1 c. c. broth culture intravenously in rabbit; no effect. 1 c. c. broth culture subcutaneously in mouse; no effect.
47	do.	+	do.	1 c. c. broth culture intravenously in rabbit; no effect. 1 c. c. broth culture subcutaneously in mouse; no effect.
63(45)	Staphylococcus.	+	Yellow	Not tested.
67(46)	Short rods, parallel arrangement, 0.5 by 1.5 micra.	+	Colorless	Broth culture 1 c. c. subcutaneously in rabbit; dead in 2 days; no lesions. 1 c. c. broth culture subcutaneously in mouse; dead in 3 days.

Attention is directed to the fact that certain of these bacteria were definitely pathogenic for animals.

It was desirable to ascertain first the resistance to phenol and to formaldehyde of certain of the organisms as compared with *B. typhosus*.

The technic was similar to that employed in the determination of the Hygienic Laboratory phenol coefficient (Hyg. Lab. Bull. No. 82) so far as manipulation was concerned, but it was modified in order to test the relative resistance of two or more organisms, instead of the relative strength of two or more disinfectants.

It was assumed that, given identical conditions, the periods of survival of cultures of two or more organisms, when exposed to the same dilutions of a single disinfectant, are an index of their relative resistance under the conditions of the experiment.

In the experiments to be reported it was found impracticable to maintain identical conditions throughout; thus, the numbers of organisms at the start of the experiments were never identical, though an effort was made to use cultures of about the same turbidity. Cultures grown in different media were used in the same test at times.

In order both to control the efficiency of the disinfectant, and to compare the resistance of the organisms from biological products, a stock culture of *B. typhosus* was included in each test.

All organisms used in the tests recorded in Table 3, and all organisms used in the tests recorded in Table 2, with the exception of *B. typhosus* and *B. coli*, were grown in meat infusion broth. In these instances, however, a meat extract broth was used. All the test organisms were planted in meat infusion broth after exposure to the disinfectant dilutions for various periods.

TABLE II.

Time required to kill certain organisms from biological products, as compared with *B. typhosus*, when exposed to a solution of 1 part phenol in 110 parts water at 20° C.

Test No.	Culture.	Age of culture.	Media.	Number of minutes exposure.								
				4	6	9	14	22	33	49	74	110
1	*B. typhosus*	24 hours	Extract broth	+	+	+	+	−	−	−	−	−
	No. 6	do	Glucose broth	+	+	+	+	−	−	−	−	−
	No. 41	do	do	+	+	+	+	−	−	−	−	−
2	*B. typhosus*	do	Extract broth	+	+	+	−	−	−	−	−	−
	B. coli, stock	do	do	+	+	+	+	+	+	+	−	−
	No. 22	do	Glucose broth	+	+	−	−	−	−	−	−	−
3	*B. typhosus*	do	Extract broth	+	+	+	+	−	−	−	−	−
	No. 47	do	Glucose broth	+	+	+	+	+	+	+	+	+
4	*B. typhosus*	do	Extract broth	+	+	+	−	−	−	−	−	−
	No. 11	do	Plain broth	+	+	+	+	+	−	−	−	−
	No. 25	do	do	+	+	+	+	+	+	+	−	−
	No. 40	do	do	+	+	+	+	+	+	+	+	+
5	*B. typhosus*	do	Extract broth	+	+	+	+	+	−	−	−	−
	No. 12	do	Plain broth	+	+	+	+	+	+	+	+	+
	No. 31	do	do	+	+	+	+	+	+	+	+	+
	No. 10	do	do	+	+	+	+	+	+	+	−	−
6	*B. typhosus*	do	Extract broth	+	+	+	+	−	−	−	−	−
	No. 13	do	Plain broth	+	+	+	+	+	+	+	+	−
	No. 14	do	do	+	+	+	+	+	+	+	−	−
	No. 67	do	do	+	+	+	+	+	+	+	+	+

TABLE III.

Time required to kill certain organisms from biological products, as compared with *B. typhosus*, when exposed to a solution of 1 part solution of formaldehyde plus 49 parts of water at 20° C. The solution of formaldehyde contained 35.82 per cent of formaldehyde gas.

Test No.	Culture.	Age of culture.	Media.	Number of minutes exposure.								
				4	6	9	14	22	33	49	74	110
1	*B. typhosus*	24 hours	Plain broth	+	+	+	+	−	−	−	−	−
	No. 10	do	do	+	+	+	+	+	+	+	+	+
	No. 12	do	do	+	+	+	+	+	+	+	+	−
	No. 41	do	do	+	+	+	−	−	+	−	−	−
	No. 47	do	do	+	+	+	+	−	−	−	−	−
2	*B. typhosus*	do	do	+	+	+	−	−	−	−	−	−
	No. 11	do	do	+	+	+	+	+	−	−	−	−
	No. 13	do	do	+	+	+	+	+	+	−	+	+
	No. 14	do	do	+	+	+	+	+	−	−	−	−
	No. 22	do	do	+	+	+	+	+	+	−	+	+
3	*B. typhosus*	do	do	+	+	+	−	−	−	−	−	−
	No. 40	do	do	+	+	+	−	−	−	−	−	−
4	*B. typhosus*	do	do	+	+	+	+	−	−	−	−	−
	No. 6	do	do	+	+	+	+	+	+	−	−	−
	No. 25	do	do	+	+	+	+	+	+	−	−	−
	No. 31	do	do	+	+	+	+	+	−	−	−	−
	No. 67	do	do	+	+	+	+	+	+	+	+	+

In the experiments just presented certain of the cultures were distinctly more resistant, both to phenol and to solution of formaldehyde than the culture of *B. typhosus*, e. g., No. 25, No. 12, No. 10, No. 13, No. 67, and No. 11. On the other hand, some of the cultures were found to be distinctly more resistant to phenol than the typhoid culture, but showed about the same resistance to solution of formaldehyde as *B. typhosus*, e. g., No. 47, No. 40, No. 31, No. 14. Again, two of the cultures were found to be distinctly more resistant to solution of formaldehyde than the typhoid culture, but showed about the same resistance to phenol as *B. typhosus*, e. g., No. 6 and No. 22.

In planning practical tests of the germicidal value of preservatives for biological products, a number of factors presented themselves for consideration. First, it was recognized that the medium in which the test organism should be exposed to the preservative should be horse serum, the vehicle for so many known or alleged curative principles in biological therapy. Accordingly this substance was always used in the tests. In addition to this, duplicate tests were carried out in plain broth. As has previously been stated, aerobic nonspore-bearing bacteria, isolated from biological products, were selected for tests organisms. The duration of the experiment was likewise an important consideration. This was usually 5, sometimes 7, days, periods practically certain to elapse between the manufacture of any biological product on a commercial scale and its use. The temperature selected for the experiments was 20° C., i. e., room temperature. In selecting this temperature it was borne in mind that while the test bacteria would have more tendency to multiply at this temperature than at ice-box temperature, the germicidal action of the preservative would probably be more pronounced at 20° C. than at a lower temperature; and it was concluded that these two factors would probably, to a certain degree, balance each other. In general, in planning the experiments recorded below, our purpose was to simulate an accidentally contaminated sample of, for example, tetanus antitoxin, and determine the efficiency of the preservative employed. It is recognized that the dose of organisms used is much larger than the mass of any single contamination in the process of manufacture or handling is likely to be. However, in this regard it was decided to err on the side of safety and to use 0.01 c. c. of a broth culture of the test organism.

The following experiments were carried out by placing accurately measured dilutions of the disinfectants, mixed with sterile horse serum and plain bouillon, in sterile test tubes, after which the tubes were inoculated with definite amounts of broth cultures and kept at 20° C. for five or seven days. Once every 24 hours a definite amount (0.1 c. c.) was removed from these test tubes and inoculated into previously heated and cooled Smith fermentation tubes, containing meat infusion broth plus 1 per cent of glucose. These were incubated for seven days at 37° C. and the presence or absence of growth at the end of that time noted and recorded (+ or — signs). No record, as a rule, was made of the appearance of the test tubes kept at 20° C. as the presence or lack of growth in the transplant was considered to be an accurate index of the presence or absence of live organisms in the 20° tubes at the time the transplant was made.

In making the transplant, especial care was taken to mix thoroughly the contents of the tubes before sampling.

As stated above, 0.1 c. c. was transplanted from the test tubes kept at 20° C. to the glucose-broth tubes which were incubated at 37° C. It seemed possible that the removal of but 0.1 c. c. of the broth or serum mixtures might fail to test accurately the presence or absence of living organisms in them; so in a number of instances, both 0.1 c. c. and 1 c. c. of the broth and serum mixtures were removed and placed in separate glucose-broth tubes. The result was that in no instance did the tube seeded with 1 c. c. show growth when the tube receiving 0.1 c. c. remained sterile.

Test tubes $\frac{5}{8}$ inch by 4 inches of clear thin glass were used. Cotton stoppers were used as a rule except in certain experiments with formalin, when sterile rubber stoppers were employed.

The disinfectant was mixed with sterile horse serum or broth (usually in 1 per cent strength) 48 hours previous to the introduction of cultures. At the end of that time, dilutions were made, using horse serum and broth respectively, and for convenience the dilutions were made up to contain 90 per cent of serum and broth, the remainder consisting of salt solution and disinfectant. The total volume in each tube was 5 c. c. Just prior to inoculation, portions of the contents of the tubes containing the least disinfectant were inocculated into fermentation tubes which were incubated and thus served to control the sterility of the materials at the start.

Ordinarily, broth cultures were used, sometimes plain broth, and sometimes glucose broth. The age of culture was 24 or 48 hours, the effort being to obtain fairly heavy growth of the organism, about the turbidity of 24-hour typhoid culture in plain broth.

Nearly always 0.01 c. c. of the broth cultures was inoculated into the tubes containing 5 c. c. of the disinfectant-serum or disinfectant-broth mixtures at the beginning of the experiment. Tests for the viability of the cultures were made in each instance. About 10 per cent of the glucose-broth fermentation tubes furnished for the experiments were incubated uninoculated, serving as controls for the sterility of the media. No growth appeared in any of these.

Table 4 shows the results on several cultures of various concentrations of trikresol (Schering).

TABLE IV.—*Trikresol as a preservative.*

Presence or absence of growth in transplants made from disinfectant mixtures on days indicated below. Mixtures inoculated 24 hours before first transplants were made.

	Trikresol.	Culture *B. typhosus* (stock culture). Days at 20° C.					Culture No. 6. Days at 20° C.					Culture No. 11. Days at 20° C.				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Per cent.															
Serum	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.6	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−
	.4	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−
	.2	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+
Broth	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.2	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+

	Trikresol.	Culture No. 12. Days at 20° C.					Culture No. 63. Days at 20° C.					Culture No. 67. Days at 20° C.				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Per cent.															
Serum	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.8	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	.6	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+
	.4	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+
	.2	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+
Broth	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.6	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
	.4	+	+	+	−	−	−	−	−	−	−	+	+	+	+	+
	.2	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+

The results of this test indicate that for any of the organisms tested, with a single exception (No. 67), 0.6 per cent trikresol is sufficient to kill in less than seven days, a period within which it is scarcely likely that a commercial preparation will reach the consumer. This strength of trikresol is greater than it is generally desirable to use, as mixtures containing this amount are likely to be turbid and unduly toxic. Four-tenths of one per cent (0.4 per cent) was effective against only three of the six organisms used in this experiment.

As was expected, the germicides were decidedly more effective in the broth mixtures than in those containing serum.

Using certain other of the cultures with 0.4 per cent trikresol we find as shown by Table 5, that in the serum mixtures three of nine survived this concentration of disinfectant for seven days, while none survived as long as three days in the broth mixtures.

TABLE V.—*Trikresol as a preservative.*

Presence or absence of growth in transplants made from disinfectant mixtures on days indicated below. Mixtures inoculated 24 hours before first transplants were made.

Serum and broth containing 0.4 per cent trikresol used in this experiment.

	Culture *B. Mallei* (stock culture). Days at 20° C.					Culture No. 8. Days at 20° C.					Culture No. 10. Days at 20° C.					Culture No. 13. Days at 20° C.					Culture No. 14. Days at 20° C.				
	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7
Serum	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Broth	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−

	Culture No. 15. Days at 20° C.					Culture No. 16. Days at 20° C.					Culture No. 22. Days at 20° C.					Culture No. 25. Days at 20° C.				
	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7
Serum	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−
Broth	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−

	Culture No. 31. Days at 20° C.					Culture No. 40. Days at 20° C.					Culture No. 41. Days at 20° C.					Culture No. 47. Days at 20° C.				
	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7
Serum	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Broth	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

So far as information at hand goes, solution of formaldehyde is not used as a preservative of biological products sold in this country, though it has been proposed for this purpose. As the following tables (Table VI and Table VII) show, unless present in such proportion that the quantity of formaldehyde is as high as 8 parts in 10,000, sterilization was not accomplished in all cases within the period of the experiment.

TABLE VI.—*Formaldehyde as a preservative.*

	Formaldehyde gas.	Culture *B. typhosus* (stock culture). Days at 20° C.					Culture No. 6. Days at 20° C.					Culture No. 11. Days at 20° C.				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Per cent.															
Serum	0.2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.08	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.04	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.02	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−
Broth	.2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.08	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.04	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	.02	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−

TABLE VI. *Formaldehyde as a preservative*—Continued.

	Formaldehyd gas.	Culture No. 12. Days at 20° C.					Culture No. 63. Days at 20° C.					Culture No. 67. Days at 20° C.				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Per cent.															
Serum	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	.12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	.08	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
	.04	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–
	.02	+	+	+	+	+	–	–	–	–	–	+	+	–	+	+
Broth	.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	.12	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	.08	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	.04	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–
	.02	+	+	+	+	+	–	–	–	–	–	+	+	–	–	–

TABLE VII.—*Formaldehyde as a preservative.*

Presence or absence of growth in transplants made from disinfectant mixtures on days indicated below. Mixtures inoculated 24 hours before first transplants were made.

Serum and broth containing 0.1 per cent commercial solution of formaldehyde used in this experiment. The solution of formaldehyde contained 35.82 per cent H-COH.

	Culture *B. mallei* (stock culture). Days at 20° C.					Culture No. 8. Days at 20° C.					Culture No. 10. Days at 20° C.					Culture No. 13. Days at 20° C.					Culture No. 14. Days at 20° C.				
	1	2	3	4	5	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7
Serum	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Broth	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–

	Culture No. 15. Days at 20° C.					Culture No. 16. Days at 20° C.					Culture No. 22. Days at 20° C.					Culture No. 25. Days at 20° C.				
	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7
Serum	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–
Broth	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–

	Culture No. 31. Days at 20° C.					Culture No. 40. Days at 20° C.					Culture No. 41. Days at 20° C.					Culture No. 47. Days at 20° C.				
	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7	1	2	3	4	7
Serum	–	–	–	–	–	+	–	–	–	–	+	+	+	+	–	+	+	+	–	–
Broth	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	+	+	–	–	–

Through the courtesy of Dr. E. K. Dunham we have been able to test experimentally one of the preparations generally indicated by the term "chloramin." As Table 8 shows, this agent is not adapted to the purpose we had in view. This substance, in the concentration used, appears to be more effective in the mixtures containing serum than in those containing broth.

TABLE VIII.

Benzene-sodium-sulphon-chloramid as a preservative. Presence or absence of growth in transplants made from disinfectant mixtures on days indicated below. Mixtures inoculated 24 hours before first transplants were made.

	Benzene-sodium-sulphon-chlor-amid.	Culture *B. typhosus* (stock culture). Days at 20° C.					Culture No. 6. Days at 20° C.					Culture No. 11. Days at 20° C.				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Per cent.															
Horse serum	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Broth	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

	Benzene-sodium-sulphon-chlor-amid.	Culture No. 12. Days at 20° C.					Culture No. 63. Days at 20° C.					Culture No. 67. Days at 20° C.				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Per cent.															
Horse serum	1	+	—	—	—	—	—	—	—	—	—	+	—	—	—	—
	.6	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+
	.4	+	+	+	+	+	+	+	+	—	—	+	+	+	+	+
	.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Broth	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

SUMMARY.

1. When certain organisms recovered from biological products where exposed to dilutions of phenol 1–110 and to solution of formaldehyde 1–50, a decided variation in the resistance of the organisms was noted. The resistance of the organisms differed both with the disinfectant used and in relation to each other and also as compared with the typhoid bacillus.

2. A method for testing the practical value of a substance for the destruction of such chance contaminating bacteria as may get into biological products has been presented.

3. Trikresol and formaldehyde, when used in amounts usually recommended and tested in serum or broth, were ineffective germicides against certain nonspore-bearing organisms previously isolated from commercial biological products.

4. Amounts of these substances large enough to have an antiseptic action against some of the organisms used in the test would probably either be deleterious to the products, or of undue toxicity.

5. These experiments emphasize the necessity for scrupulous care in the preparation and handling of biological products and demonstrate the fact that the use of preservatives does not insure the sterility of the finished products.

ACKNOWLEDGMENTS.

The writer is indebted for the description of the cultures to Miss Ida A. Bengtson, and for the performance of the tests of the comparative resistance of the test organisms to Miss Rose Parrott, both of the laboratory staff.

THE EFFECT OF ETHER ON TETANUS SPORES AND ON CERTAIN OTHER MICROORGANISMS

By H. B. Corbitt, Sanitary Bacteriologist, U. S. Public Health Service.

In order to hasten the reduction in the number of living bacteria which occurs when ground vaccine virus is exposed to the action of glycerin it has been recommended that the vaccine be treated with ether vapor. There is no doubt that this procedure does result in rapidly lowering the counts, but there is some question as to whether it does not lead to a reduction of the potency of the specific virus at the same time.

As it is highly desirable to have a method whereby any spores of tetanus which may have gained access to vaccine virus could be killed, the following experiments were undertaken. In the course of extensive examinations at the Hygienic Laboratory, covering a period of many years, tetanus organisms have never been found in vaccine virus nor has any case of post vaccinal tetanus been traced to the virus, still those engaged in the manufacture and control of vaccine virus are constantly concerned about the possibility of such an accident.

While it would be undesirable to treat vaccine virus according to the method used below, it was assumed that if spores were not killed under these conditions they would not be killed at the lower temperatures and under the other conditions less favorable for germicidal action which surround the ether treatment of the virus. The conditions of the experiment were much more favorable to the action of the ether than they ever would be in practice.

The apparatus used for the purpose of this experiment was assembled in the following manner: A water bath used for heating the ether was kept at 40° C.–42° C. by means of an electric thermo regulator. The ether and spores were placed in a Drexel bottle, in the mouth of which was inserted vertically a Liebig condenser. The continuous dropping of the condensed ether gave some agitation to the liquid in the container.

Five c. c. of a 7-day glucose broth culture of tetanus (culture No. K–27) and 20 c. c. of ether were used. Spores were found on microscopic examination of the culture. The treated culture and also a portion of the supernatant ether were planted separately in freshly heated glucose broth fermentation tubes. In all tests tetanus was recovered, in one case after 48 hours' exposure.

A check on the efficacy of the apparatus was obtained from the results on nonspore-forming organisms, which were readily killed by this treatment.

In order to test the effect of a more intimate mixture of ether and tetanus spores the following apparatus was assembled: A small water motor was connected with and turned a 5-inch wheel, to which was attached a bottle containing the broth culture of tetanus spores and ether. This was revolved at about 40–45 revolutions per minute. A thick, viscous, homogeneous emulsion resulted. The broth culture settled out very slowly. For this reason both broth and ether were planted. Tetanus organisms were recovered after 14 days' exposure. The efficacy of this apparatus was tested by the treatment of nonspore-bearing organisms under the same conditions.

THE EFFECT OF ETHER ON NONSPORE-FORMING ORGANISMS.

The following organisms were subjected to treatment with ether: *Staphylococcus aureus*, *Bacillus typhosus*, and a Gram positive rod isolated from serum and known to be rather resistant to germicides of the phenol group. To date spores have not been demonstrated in culture of this organism.

The staphylococcus was found to have been killed under three hours when the first planting was made, *B. typhosus* under 5 minutes, while the Gram positive rod failed to give growth after 11 days, but was viable at the end of 9 days.

SUMMARY OF RESULTS AND CONCLUSIONS.

1. A treatment of tetanus spores with ether at 40° C. for 48 hours failed to kill them.

2. Intimate mixing of ether and tetanus spores up to two weeks failed to kill the spores.

Therefore, we may safely assume that ether cannot be depended on to kill tetanus spores in vaccine virus.

DO NOT DESTROY.
When you have no further use for this pamphlet, give it to someone else.

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TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY—BULLETIN No. 113
JULY, 1918

I. AN EXPERIMENTAL INVESTIGATION OF THE TOXICITY OF CERTAIN ORGANIC ARSENIC COMPOUNDS

By GEORGE B. ROTH

II. ON THE TOXICITY OF EMETINE HYDROCHLORIDE, WITH SPECIAL REFERENCE TO THE COMPARATIVE TOXICITY OF VARIOUS MARKET PREPARATIONS

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I. AN EXPERIMENTAL INVESTIGATION OF THE TOXICITY OF CERTAIN ORGANIC ARSENIC COMPOUNDS.[1]

By George B. Roth.

(From the Division of Pharmacology, Hygienic Laboratory, United States Public Health Service, Washington, D. C.)

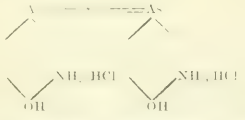
PART I. SALVARSAN MADE IN AMERICA.

INTRODUCTION.

Previous to the present war salvarsan [2] was exclusively a German product. Shortly after the outbreak of the war importation of salvarsan into the United States was stopped and, as the available supply was limited, it soon became practically impossible to obtain German-made salvarsan. To prevent the United States from being deprived of this valuable remedy, certain investigators in this country immediately took up the question of its manufacture and after a period of investigation placed upon the market, through the American agents of the German manufacturer of salvarsan, a substance which is practically identical with the German product.

About the same time that investigations were begun in the United States, similar studies were made in England, France, Canada, and Japan, which resulted in the production of compounds which are now used as substitutes for the original.

Salvarsan is the dihydrochloride of 3–3′ diamino 4–4′ dioxy-arsenobenzene. Its structural formula as given by Ehrlich and Bertheim (1912), is as follows:



It is also known as kharsivan (England), arsenobenzol (France), arsenobenzol and salvarsan (United States), diarsenol (Canada), and arsaminol (Japan).

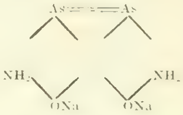
---

[1] Manuscript received for publication Jan. 24, 1918.

[2] The name salvarsan as used in this paper refers to the arsenic compound 3–3′ diamino 4–4′ dioxy-arsenobenzene dihydrochloride.

The substance was first prepared by Bertheim in 1910 at the suggestion of Ehrlich, and was given the name "606" from the laboratory number of the preparation. It was later shown, by Ehrlich and others, to be a specific in combating infection with the organism of syphilis. It is unlike certain other organic preparations of arsenic in that it contains the arsenic in the trivalent form. The arsenic content of salvarsan averages about 30 per cent.

Salvarsan is a yellow powder which dissolves quite readily in hot water, making an acid solution. Aqueous solutions of salvarsan, either acid or alkaline, are very unstable. According to Ehrlich and Bertheim (1912), the powder itself is very readily oxidized in the air and forms amino-oxyphenylarsenic oxide which is (according to these investigators) about 20 times as toxic as salvarsan. When salvarsan is made alkaline with sodium hydroxide it forms the sodium salt of dioxydiamino-arsenobenzene, which, according to Ehrlich and Hata (1910), has the following structural formula:



Theoretically, the arsenic in salvarsan should exist in the organic form only. It has been found, however, that regardless of the fact that the utmost care may have been taken in the manufacture of salvarsan, a certain variable amount of arsenic will be present in the inorganic form. In this latter form arsenic is many times more toxic than in the form of salvarsan. It has been shown, furthermore, that at times certain organic arsenic compounds which possess a higher toxicity than salvarsan may enter into the composition of the finished product. Chemical analysis will not reveal the presence of these more toxic products and it is therefore essential to determine the toxicity of salvarsan by biologic tests before it is placed on the market.

A few reports have already been made relative to the biological effects of American-made salvarsan. The investigations of Wile (1916), of Ormsby and Mitchell (1916), and of Moody (1916) show that the salvarsan made in the United States is no more toxic clinically than the original German product; while the report of Gardner (1916) would indicate that salvarsan made in Canada is a true substitute for German salvarsan. In addition to the above-named clinical papers there has recently appeared a report of a very extensive laboratory investigation by Schamberg, Kolmer, and Raiziss (1917). These observers corroborated the findings of Wile (1916) and others by means of biological tests carried out in the laboratory.

The salvarsan made in America, obtainable at the time of this investigation,[1] was limited to three products, namely: (1) Arsenobenzol, which is manufactured by the Dermatological Research Laboratories, of Philadelphia; (2) diarsenol, a product made by the Synthetic Drug Co., of Toronto, Canada; and (3) salvarsan, which is manufactured by the Farbwerke-Hoechst Co., at the H. A. Metz Laboratories, of New York.

Inasmuch as requests have recently been made for information regarding the toxicity of American-made salvarsan, it was deemed advisable to carry out certain studies which would elucidate this point. Toxicity studies on rabbits, rats, and guinea pigs therefore were made with arsenobenzol (Dermatological Research Laboratories, of Philadelphia), diarsenol (Synthetic Drug Co., Toronto), and of salvarsan (Metz Laboratories). These products were compared with authentic samples of German salvarsan.

DESCRIPTION OF SAMPLES OF SALVARSAN EXAMINED.

The samples of arsenobenzol examined were obtained in June, 1917, through the American agent of the Farbwerke-Hoechst Co., who at that time acted as the distributing agent for this product. Two lots were examined, bearing the lot numbers 257 and 271, respectively. One lot of the diarsenol which was examined was obtained in June, 1917, directly from the Synthetic Drug Co. The 10 individual ampoules which were received bore the letter A and were numbered from 32790 to 32799, inclusive. The second lot of diarsenol was received in November, 1917, directly from the manufacturers. The 12 individual ampoules were numbered B72900 to B72911, inclusive. The salvarsan made by the Metz Laboratories was received in September, 1917, and was obtained directly from the manufacturer. It was designated as lot A2.

One lot of German salvarsan, with which the American-made salvarsan was compared, was sent to the director of the Hygienic Laboratory in 1913 and bore no lot number. The second lot was said to be part of a consignment received from Germany a short time before the entrance of the United States into the war, and was obtained from the American agent of the Farbwerke-Hoechst Co. in July, 1917. It bore the lot number A28001

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[1] This investigation was practically completed when the report of the Federal Trade Commission relative to the manufacture of salvarsan in America appeared. On Nov. 30, 1917 (see Public Health Reports, 1917, vol. 32, p. 2071), the Federal Trade Commission issued orders for licenses to manufacture and sell the product heretofore known under the trade names of "Salvarsan," "606," "Arsenobenzol," and "Arsaminol," to the following manufacturers: Farbwerke-Hoechst Co. (Herman A. Metz Laboratory), of New York; Dermatological Research Laboratories, of Philadelphia; and the Takamine Laboratory (Inc.), of New York The drug is to be manufactured and sold under the name of "Arsphenamine."

COLOR, REACTION, AND SOLUBILITY OF THE VARIOUS PRODUCTS INVESTIGATED.

All of the preparations examined were put up in sealed glass ampoules and consisted of a light canary-yellow powder. The samples of arsenobenzol and diarsenol were of a slightly deeper yellow than the German salvarsan and therefore could be distinguished from the others by this difference in color alone. However, a sample of arsenobenzol recently received but not examined could not be distinguished from the German product in this way.

Every preparation was acid to litmus and required approximately the same amount of sodium hydroxide to make it distinctly alkaline to litmus, that is, about 1.2 c. c. of a 15 per cent solution for each 0.6 gm. of material.

Arsenobenzol was somewhat less soluble in hot water than the other preparations. While no accurate quantitative methods were used in determining the solubilities of these preparations, it appeared that the 1913 sample of German salvarsan was slightly more soluble in hot water than any of the others.

In order that the results of the present investigation may be readily compared with the results obtained by previous investigators, an epitome of the literature relating to the determination of the toxicity of salvarsan by a similar laboratory method is given in Table 1. The table relates only to the results obtained from the intravenous administration of an alkaline solution of the drug to rabbits. No data are available with reference to the toxicity of the drug for guinea pigs, when administered by the intravenous route: while for rats, only two references were found. Schamberg, Kolmer and Raiziss (1917), carried out an extensive series of experiments upon rats, the drug being injected through the exposed jugular vein, and found that these animals could tolerate arsenobenzol (Phila. Dermatological Research Laboratory) in amounts ranging from 0.070 to 0.100 gm. per kilo of body weight. The drug was dissolved in 1 c. c. of water. Hata and Hirano (1916) determined the tolerated dose of arsaminol for rats, when given intravenously, as 0.1 gm. per kilo of body weight.

TABLE 1.—*Epitome of literature relating to the toxicity of salvarsan when given intravenously to rabbits as an alkaline aqueous solution.*

Observer.	Where reported.	Preparation.	Dose per kilo.	Remarks.
Castelli.	Ztschr. f. Chemotherap. Orig. 1912-13, p. 321.	Salvarsan (German).	Dosis tolerata 0.1 gm.	
Ehrlich and Hata.	Chemotherapie der Spirillosen, 1910, p. 23.	do.	do.	
Hata and Hirano.	Saikin Gaku-Zasshi, 1916, No. [illegible], p. 321.	Salvarsan (German) and arsaminol (Japan).	M. L. D. 0.1 gm. of either preparation.	Observation period, weeks.
[illegible]	Ztschr. f. exp. Path. u. Therap. 1911, vol. 9, p. 332.	Salvarsan (German).	M. L. D. 0.204 gm.	Given through jugular vein as a 0.5 per cent solution in 0.9 per cent saline.

TABLE 1. —*Epitome of literature relating to the toxicity of salvarsan when given intravenously to rabbits as an alkaline aqueous solution*—Continued.

Observer.	Where reported.	Preparation.	Dose per kilo.	Remarks.
Hoppe and Schreiber	Verhandlung. Deutsch Kongress f. Innere Med. 1910, p. 243.	do.	Toxic dose (M. L. D.) 0.15 gm.	
Kersten	Centralbl. f. Bakt. 1912, vol. 65, p. 369 (Orig.).	do.	Dosis tolerata 0.075 gm. (ave).	0.1 gm. killed 50 per cent in 1 to 2 days; given as 1 or 2 per cent solution
Kochmann.	Münch. Med. Wchnschr. 1912, vol. 59, p. 18.	do.	M. L. D. (delayed death): 0.1 gm.; M. L. D. (acute) 0.2 gm.	Perfused slowly as 2 to 3.5 per cent solution 0.1 gm. killed in 9 days 0.2 gm. killed in 2 hours Observed for 18 days
Marschalkó and Veszprémi.	Deutsch. Med. Wchnschr. 1912, vol. 38, p. 1222.	do.	Dosis tolerata less than 0.1 gm.	Given in saline as a 1 per cent solution.
Pearce and Brown.	J. Pharmacol. and Exper. Therap. 1917, vol. 9, p. 354.	do.	M. L. D.: 0.112 gm.	M. L. D. includes death either from acute or chronic poisoning. Injection rate 2 c. c. per minute, given as a 1 per cent solution. (Personal communication.)
Schamberg, Kolmer and Raiziss.	Jr. Cutaneous Dis. 1917, vol. 35, p. 286.	Arseno-benzol (Phila. Derm. Research Lab.)	Tolerated dose 0.060 gm.	Injection time several minutes; wait of 2 minutes after one-half was given. Given in 15 c. c. distilled water. Animal should live 2 weeks.
Willcox and Webster.	Brit. M. J. 1916, vol. 1, p. 473.	Kharsivan (English salvarsan).	Tolerated dose 0.1 gm.	Given as a [illegible] per cent solution.

Some of the observers quoted in this table of the literature on salvarsan used acid as well as alkaline solutions of the drug, and found that the acid solution was considerably more toxic for rabbits when given intravenously than was the alkaline solution. Similar investigations on the toxicity of the acid solution of salvarsan for rabbits were made by Hering (1910), Auer (1911), and Miedreich (1913).

ARSENIC CONTENT OF THE SALVARSAN PREPARATIONS EXAMINED.

The arsenic content of the salvarsan preparations[1] used in this investigation was determined by the method described by Ewins (1916), and is shown in the following table:

TABLE 2.

Preparation.	Manufacturer's or laboratory No.	Arsenic content (per cent).
Salvarsan (German)	1913 sample	29.5
Do.	1917 sample (A28001)	31.7
Salvarsan (Metz Laboratory)	A2	30.1
Arsenobenzol (Phila. Derm. Research Lab.)	257	30.7
Do.	271	30.9
Diarsenol (Synthetic Drug Co.)	A32798	29.8
Do.	B72911	29.8

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[1] I am indebted to Dr. C. N. Myers of the Hygienic Laboratory for the determination of the salvarsan preparation made by the Metz Laboratory and to Dr. A. Seidell, also of the Hygienic Laboratory, for the determination of all others.

The above table shows that the arsenic content of the preparations examined ranges from 29.5 to 31.7 per cent and that, for all samples it averages 30.4 per cent. It is furthermore shown that the greatest variation is between the two German samples examined, the difference being over 2 per cent.

TEST ANIMALS EMPLOYED.

Healthy rabbits, rats, and guinea pigs were employed as test animals in the present investigation. The rabbits were obtained from various sources and consisted largely of the short-haired Belgian type, although many white, black and mixed types were used. An attempt was made to secure rabbits of a weight ranging between 1.2 and 1.8 kilos, but it was found impossible to secure invariably animals which met the above weight requirements.

The rats were, in practically all cases ordinary albino rats; in some instances they were of a mixed type and presented a mottled coating of white and black hair. The rats were obtained for the most part from the Wistar Institute of Philadelphia and from local dealers.

The guinea pigs were obtained from fresh Hygienic Laboratory stock.

In these experiments the animals were confined in suitable laboratory cages and were fed the usual laboratory diet, which previous experience had shown to be adequate.

Length of observation period of animals. Although the animals were practically all kept for one month before being discharged, it was considered that a two weeks' period was sufficient, and perhaps best, as a time limit for the estimation of the minimal lethal dose. This time limit was chosen largely because it was shown by Burnascheff (1912) that when salvarsan was given to rabbits intravenously in alkaline solution, only traces of arsenic could be found in the various organs of the body after three weeks. Furthermore, a two weeks' period obviates other possible factors, such as decreased resistance, due to prolonged confinement under laboratory conditions.

In all the following tables, if death did not occur within two weeks, or, if it occurred later than two weeks after the injection, the result is designated by a minus sign (–). This sign therefore means that the animal lived for two weeks or longer. If death occurred before the two weeks had elapsed, the result is designated by a plus sign (+).

Method of administering the drugs.—The drugs were administered intravenously in all cases, in order not only to preclude any loss of the drug by nonabsorption but to eliminate differences in the rate of absorption, such as may occur when the drug is given subcutaneously; moreover intravenous administration is the method usually employed in therapeutics. In the rabbit, injections were made in the marginal ear vein; in the rat, the large superficial vein of the inner side of the hind leg was employed in practically all of the experiments, although in a small number of instances, one of the superficial veins at the base of the tail was used. In the guinea pig, one of the superficial veins of the outer side of the front leg was used.

The vessel used in the rat and guinea-pig was exposed by a small skin incision; by compressing it beyond the incision the injection of the drug was rendered simple. An anesthetic was neither required nor used in any of the experiments. To prevent hemorrhage in the guinea pig and rat a small compress of cotton was placed between the skin and the vessel; this was removed, usually after a day or two. If this procedure did not prevent bleeding, the vessel was ligated. Bleeding was prevented in the rabbit by means of an improvised wire clamp.

For measuring the doses required for rats and guinea pigs, glass syringes of 1 or 2 c. c. capacity were used. The same syringe which was used for measuring the drug was also used for its injection into these animals. The syringes had been standardized by the manufacturers, but before being used they were standardized in the laboratory against each other.

In the earlier experiments the drugs were given to rabbits by means of glass syringes of 5 to 10 c. c. capacity; while in the later experiments a burette of 25 c. c. capacity was used. Injections were made in rabbits by means of syringes, or from a burette by gravity.

The same syringes, cylinders, and burette were used throughout the investigation.

MANNER OF MAKING THE SALVARSAN SOLUTION.

The solution of salvarsan was made up as follows unless otherwise stated. To make a 1 per cent solution of salvarsan an ampoule containing 0.6 gm. of the drug[1] was dissolved in a 100 c. c. glass-stoppered cylinder which contained about 50 c. c. of hot water (recently redistilled in glass). The distilled water had previously been sterilized by boiling and was transferred to the cylinder immediately thereafter, so that the solution of the salvarsan was made in water that had a temperature only a little lower than the boiling point. After a complete solution of the drug through vigorous shaking, a 15 per cent solution of sodium hydroxide was added drop by drop (shaking after the addition of each drop or two) until the solution was slightly alkaline. This required about 1.2 c. c. of the sodium hydroxide solution. Sterile distilled water was then added to make a 1 per cent solution. When prepared in the above manner the solutions were always clear and did not require filtration.

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[1] The salvarsan was invariably weighed on an analytical balance previous to making the solution. The weight was found to be practically as stated. In a few instances a variation of about 10 mg., either greater or less than stated, was found.

CONCENTRATIONS EMPLOYED AND RATE OF ADMINISTRATION.

The salvarsan preparations were given intravenously as 1 or 2 per cent alkaline aqueous solutions. They were given to rats and guinea pigs at the rate of about 0.5 to 0.7 c. c. per minute (leg vein), while to rabbits the rate was increased to about 1 c. c. per minute (ear vein). If the injections were made at the above rate immediate symptoms rarely resulted. If immediate symptoms occurred the injection was stopped until their disappearance and then resumed.

LENGTH OF TIME SOLUTIONS STOOD BEFORE BEING USED.

The injections were almost invariably made as soon as possible after making up the solution. Except in one instance, a solution was never used for toxicity tests after it was more than three hours old. In that instance it was tested 5 and 6 hours after making, in order to determine whether it had deteriorated.

ANTISEPTIC METHODS OBSERVED.

The animals were prepared for the injection of the drugs by shaving the hair over the site of the skin incision and then painting the skin with tincture of iodine, or washing it with 70 per cent alcohol. All instruments were sterilized by boiling.

TABLE 3. *Toxicity of salvarsan, German (1915 sample), when given intravenously to rabbits (ear vein) as a 1 per cent alkaline aqueous solution.*

No. of animal	Sex: M.=male; F.=female.	Weight in kilos	Dose per kilo.	Result: +=dead; −=living.	Time of death or discharge.
			Mgm.		
69	F	1.61	60		Died 6 d.
70	F	1.62	60		Disch. 32 d.
71	F	1.66	60		Do.
75	M.	1.54	60		Died 13 d.
68	M	1.52	100		Disch. 33 d.
73	F	1.53	100		Do.
77	F	1.94	100		Died 4 d.
79	F	1.77	100		Do.
72	F	1.59	175		Died 2 d.
74	F	1.59	175		Died 1 hr.
76	F	1.56	175		Died [illegible] d.
78	M	1.71	175		Died 3 d.

Weight variation, 1.52 to 1.94 kilos.
Average weight, 1.64 kilos.
Variation in amount of fluid injected, 9.3 to 29.9 c. c.

TABLE 4.—*Toxicity of salvarsan, German (1913 sample), when given intravenously to rats (leg vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead: −living.	Time of death or discharge.
			Mgm.		
131.	M	170	50	–	Disch. 31 d.
132	M	122	50	–	Do
137..	F..	110	50		Do.
138.	M	138	50	–	Do.
78..	M..	178	60	–	Do.
83..	F	124	60	–	Do.
89...	F	169	60	–	Disch. 30 d
94..	M...........	135	60	–	Do.
133...	M...........	121	70	–	Disch. 31 d.
134...	F..	170	70	+	Died 18 hrs.
139...	F...........	158	70	–	Disch. 31 d.
140...	F...........	122	70	–	Do.
79.....	F...........	130	80	+	Died 4 d.
86...	F...........	139	80	+	Died 15 hrs.
90....	F.	169	80	–	Disch. 30 d.
95...	M.	140	80	–	Do.
135......	M...........	170	90	+	Died 18 hrs.
136...	F.	122	90	+	Do.
141....	F.....	142	90	–	Disch. 31 d.
142....	M.	138	90	–	Do.
80..	F...........	122	100	+	Died 6 d.
84...	F...........	118	100	+	Died 4 d.
91..	F...........	179	100	–	Disch. 30 d.
96..	M.	116	100	–	Do.
81..	M....	116	135	+	Died 15 hrs.
87...	M.	130	135	+	Do
92.......	F...........	166	135	+	Died 5 hrs.
97....	F...	134	135	+	Died 15 hrs.
82......	F...........	104	175	+	Do.
85....	F..	110	175	+	Do
93..	F...........	147	175	+	Died 1 min.
98..	M.	105	175	+	Died 5 hrs.

Weight variation, 104 to 179 gm.
Average weight, 138 gm.
Variation in amount of fluid injected, 0.55 to 2.57 c.c.

TABLE 5.—*Toxicity of salvarsan, German (1917 sample A 28001), when given intravenously to rabbits (ear vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
106	M	1.43	60	–	Disch. 31 d.
107.	M.	1.5	60	+	Died 6 d.
109	F	1.52	60		Disch. 31 d.
112.	F	1.77	60		Do.
105.	F	1.79	80		Do.
108.	M	1.59	80	+	Died 7 d.
115.	F	1.77	80		Died 21 d.
116	F	1.82	80		Died 7 d.
110.	F	1.7	100		Disch. 32 d.
111	F	1.52	100		Died 7 d.
113	M	1.57	100	+	Died 8 d.
114..	F	1.69	100		Disch. 31 d.
117	F	1.48	135	+	Died 6 hrs.
118.	F	1.39	135	+	Died 3 d.
119	F	1.44	135	–	Disch. 31 d.
120.	F	1.52	135	+	Died 6 hrs.
121	F	1.2	175	+	Died 2 d.
122.	F	1.48	175	+	Died 6 d.
123.	F	1.43	175	+	Died 2 d.
124	F	1.18	175	+	Died 3 d.

Weight variation, 1.18 to 1.82 kilos.
Average weight, 1.54 kilos.
Variation in amount of fluid injected, 8.6 to 25.9 c.c.

TABLE 6.—*Toxicity of salvarsan, German (1917 sample A28001), when given intravenously to rats (leg vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male. F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
155	F	194	50		Disch. 31 d.
156	M	192	50		Do.
161	F	140	50		Disch. 36 d.
162	F	192	50		Died, 2 d.
157	F	170	60		Disch. 31 d.
165	F	140	60		Disch. 35 d.
166	M	150	60		Disch. 36 d.
167	F	140	60		Died 14 hrs.
168	M	152	70		Disch. 33 d.
174	M	118	70		Do.
169	F	140	70		Do.
188	M	140	70		Disch. 32 d.
158	M	190	76		Died 1 hr.
159	F	116	80		Disch. 31 d.
160	M	184	80		Disch. 36 d.
163	F	150	80		Died 14 hrs.
164	M	162	80		Do.
170	M	142	90		Disch. 33 d.
171	M	142	90		Do.
175	M	120	90		Do.
189	M	140	90		Died 8 min.
172	M	122	100		Disch. 33 d.
173	M	120	100		Do.
190	M	132	100		Died 5 hrs.
191	M	134	100		Disch. 32 d.
194	F	116	110		Died 4 hrs.
176	M	124	135		Died 22 d.
178	M	128	135		Died 14 min.
192	M	130	135		Died 10 min.
193	M	118	135		Died 2 hrs.
177	F	122	175		Died 45 min.
179	F	110	175		Died 5 hrs.
195	F	116	175		Do.
196	F	106	175		Died 2 hrs.

Weight variation, 106 to 194 gm.
Average weight, 141 gm.
Variation in amount of fluid injected, 0.7 to 2.1 c. c.

TABLE 7.—*Toxicity of salvarsan, German (1917 sample A28001), when given intravenously to rats (leg vein) as a 2 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
443	M	112	50	−	Disch. 30 d.
444	M	110	50	−	Do.
445	M	112	50	−	Do.
446	F	140	50	+	Died 4 hrs.
439	M	100	60	+	Died 10 d.
440	F	100	60	−	Disch. 30 d.
441	M	114	60	−	Do.
442	M	100	60	−	Died 20 d.
435	M	104	70	−	Disch. 30 d.
436	M	102	70	+	Died 4 hrs.
437	M	104	70	+	Died 5 hrs.
438	M	110	70	+	Died 7 hrs.
412	M	122	80	−	Disch. 30 d.
413	M	128	80	+	Died 7 hrs.
414	M	136	80	−	Disch. 30 d.
415	M	140	80	−	Do.
411	M	110	89	+	Died 2 hrs.
420	M	110	90	+	Died 5 hrs.
421	M	110	90	+	Died 3 hrs.
422	M	110	90	+	Do.
407	M	140	100	+	Died 40 min.
408	M	125	100	+	Died 3 hrs.
409	F	150	100	+	Died 20 min.
410	F	150	100	+	Died 10 min.
416	M	120	135	+	Died 1 hr.
417	M	120	135	+	Died 20 min.
418	M	110	135	+	Died 10 min.
419	M	110	135	+	Died 5 min.

Weight variation, 100 to 150 gm.
Average weight, 118 gm.
Variation in amount of fluid injected, 0.28 to 0.81 c. c.
Remarks.—Rat No. 446 was somewhat emaciated when the drug was given.

TABLE 8.—*Toxicity of salvarsan (Metz Laboratory, lot A2), when given intravenously to rabbits (ear vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			mgm.		
140	F	1.4	60		Disch. 31 d.
141	M	1.2	60	—	Died 24 d.
142	F	1.5	60	+	Died 10 d.
143	F	1.45	60	—	Disch. 31 d.
149	F	1.5	80		Do.
150	M	1.5	80	+	Died 4 d.
152	M	1.55	80	—	Disch. 31 d.
151	M	1.65	82	+	Died 9 d.
137	F	1.25	100	+	Died 3 d.
138	F	1.4	100	+	Died 2 d.
139	M	1.35	100		Disch. 31 d.
144	M	1.6	100	-	Died 10 d.
153	F	1.7	135	-	Died 3 d.
154	M	1.55	135	+	Do.
155	F	1.35	135	-	Do.
156	F	1.4	135		Died 2 d.
145	F	1.4	175	-	Died 6 hrs.
146	F	1.25	175	+	Died 2 d.
147	M	1.45	175	-	Died 4 hrs.
148	M	1.45	175	+	Do.

Weight variation, 1.2 to 1.7 kilos.
Average weight, 1.44 kilos.
Variation in amount of fluid injected, 7.2 to 25.4 c. c.

TABLE 9.—*Toxicity of salvarsan (Metz Laboratory, lot A2), when given intravenously to rats (leg vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			mgm.		
260	F	140	50		Disch. 31 d.
261	M	130	50		Do.
270	F	120	50		Do.
271	M	120	50		Do.
262	F	130	60	+	Died 20 min.
263	F	130	60		Disch. 31 d.
272	M	110	60		Do.
273	F	110	60		Do.
276	M	112	63	+	Died 1 min.
274	M	110	70	+	Died 35 min.
275	F	110	70		Died 17 min.
285	M	120	70	+	Died 30 min.
286	F	120	70	+	Died 2 d.
264	F	128	80	+	Died 1 min.
265	M	130	80		Disch. 31 d.
282	F	112	80		Do.
277	M	110	80	+	Died 3 hrs.
278	M	130	90		Disch. 31 d.
279	M	135	90	+	Died 6 hrs.
287	M	115	90	+	Died 2 hrs.
288	F	118	90		Disch. 31 d.
266	F	125	100		Died 3 d.
267	M	124	100	+	Died 30 min.
280	M	120	100	+	Died 17 hrs.
281	M	120	100	+	Died 25 min.
268	M	120	115	+	Died 35 min.
269	M	120	115	+	Died 1 min.
283	M	110	115	+	Died 20 min.
284	F	110	115	+	Died 10 min.
289	M	115	135	+	Died 5 hrs.
290	F	112	135	+	Died 9 hrs.
291	M	110	135	+	Died 4 hrs.
292	F	112	135	+	Died 5 hrs.

Weight variation, 110 to 140 gm.
Average weight, 119 gm.
Variation in amount of fluid injected, 0.6 to 1.55 c. c.

TABLE 10.—*Toxicity of salvarsan (Metz Laboratory), Lot A, when given intravenously to rats (leg vein) as a 2 per cent alkaline aqueous solution.*

No. of animal.	Sex M=male. F=female.	Weight in grams.	Dose per kilo.	Result +dead; −living.	Time of death or discharge.
			mgm		
378	F	148	50		Died 10 d.
379	F	142	50		Disch. 31 d.
380	F	128	50		Do.
381	M	124	50	—	Do.
359	M	110	60	—	Do.
360	F	110	60	-	Do.
361	F	120	60	+	Died 5 d.
362	F	120	60		Disch. 31 d.
374	F	130	70	+	Died 4 hrs
375	F	138	70	+	Died 5 d.
376	F	102	70		Disch. 31 d.
377	F	100	70	+	Died 10 d.
363	F	130	80	+	Died 2 d.
364	F	108	80	+	Died 11 min
365	F	130	80		Disch. 31 d
366	F	108	80	+	Died 25 min
370	M	114	90	+	Died 5 min
371	M	118	90	+	Died 2 d
372	F	112	90	-	Disch. 31 d.
373	F	108	90	—	Do.
355	M	150	100	+	Died 50 min.
356	F	160	100	+	Died 6 min
357	F	102	100	+	Died 30 min.
358	F	102	100	+	Died 1 hr.
367	F	150	112	+	Died 4 min.
368	F	[illegible]	135	+	Died 10 min.
369	F	112	135	+	Died 40 min.
382	F	110	135	+	Died 3 hrs.
383	F	110	135	+	Died 30 min.

Weight variation, 100 to 160 gm.
Average weight, 123 gm.
Variation in amount of fluid injected, 0.31 to 1.08 c. c.

TABLE 11.—*Toxicity of arsenobenzol (lot No. 257, Philadelphia Dermatological Research Laboratory) when given intravenously to rabbits (ear vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			mgm		
80	F	1.55	60	—	Disch. 30 d.
81	M	1.47	60		Do.
82	F	1.64	60	-	Do.
87	F	1.71	60		Disch. 31 d.
93a	F	1.53	60		Do.
88	M	1.38	80	+	Died 7 d.
89	F	1.38	80	—	Disch. 31 d.
90	M	1.54	80	—	Disch. 30 d.
91	F	1.4	80	+	Died 9 d.
83	F	1.42	100	—	Died 19 d.
84	F	1.7	100	+	Died 4 d.
85	F	1.67	100	+	Do.
86	M	1.5	100	+	Died 10 d.
95	F	1.38	100	-	Disch. 30 d.
92	F	1.54	135	+	Died 1 min.
93b	M	1.45	135	+	Died 3 d.
94	F	1.36	135	+	Died 2 hrs
96	F	1.35	135	+	Died 7 d.
97	F	1.2	175	+	Died 3 d.
98	M	1.16	175	+	Do.
99	F	1.13	175	+	Died 2 d.
104	F	1.66	175		Disch. 31 d.

Weight variation, 1.13 to 1.71 kilos.
Average weight, 1.46 kilos.
Variation in amount of fluid injected, 8.8 to 20 c. c.

Remarks.—The dose given to animal 104 was over twice as large as that given to 88 and 91, yet 104 survived, whereas 88 and 91 died within 2 weeks. Rabbit 89 developed, after 15 hours, slight swelling at the base of the injected ear. Rabbit No. 104 had a slight blood stained exudate in both ears when discharged.

TABLE 12.—*Toxicity of arsenobenzol (lot No. 257, Philadelphia dermatological Research Laboratory) when given intravenously to rats (leg vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
119	M	160	50		Disch. 31 d.
120	M	142	50		Do.
123	F	150	50		Disch. 30 d.
124	M	122	50		Do.
99	M	143	60	−	Disch. 31 d.
104	M	142	60	+	Died 3 d.
106	M	127	60	−	Disch. 30 d.
301	M	120	60		Do.
121	M	145	70		Disch. 31 d.
122	M	124	70	−	Do.
125	F	155	70		Disch. 30 d.
126	M	130	70		Died 20 d.
110	F	130	75	+	Died 3 hrs.
100	F	148	80		Died 11 hrs.
105	M	151	80		Disch. 30 d.
111	M	132	80	+	Died 7 d.
112	M	130	80	+	Died 21 hrs.
127	F	150	90	+	Died 20 min.
128	M	112	90	+	Died 1 hr.
129	M	122	90	+	Died 18 hrs.
130	F	145	90	+	Died 6 hrs.
101	M	115	100	+	Died 4 hrs.
107	F	156	100	+	Died 42 min.
113	M	150	100	+	Died 11 min.
115	F	134	100	+	Died 13 min.
102	F	135	135	+	Died 2 hrs.
108	M	136	135	+	Died 40 min.
114	F	130	135	+	Died 7 min.
116	F	124	135	+	Died 10 min.
117	F	112	175	+	Died 14 min.
103	F	118	175	+	Died 1 min.
109	F	125	175	+	Do.
118	M	112	175	+	Died 11 min.

Weight variation, 112 to 160 gm.
Average weight, 134 gm.
Variation in amount of fluid injected, 0.61 to 2.18 c. c.

TABLE 13.—*Toxicity of arsenobenzol (Lot No. [illegible], Philadelphia Dermatological Research Laboratory) when given intravenously to rat[illegible] [illegible] as a [illegible] percent alkaline aqueous solution.*

No. of animal.	Sex: M = male; F = female.	Weight in grams.	Dose per kilo.	Result: + dead; — living.	Time of death or discharge.
			Mgm.		
310	F	122	60	+	Died 3 hrs.
311	M	130	60		Disch. 30 d.
312	M	134	60		Do.
313	M	132	60	—	Do.
319	M	134	70	—	Do.
320	M	112	70	—	Do.
321	M	122	70	—	Do.
322	M	120	70	—	Do.
323	M	140	80		Do.
324	M	140	80		Do.
325	M	146	80		Do.
326	M	144	80	—	Do.
306	M	125	90	+	Died 30 min.
307	M	130	90	+	Died 5 hrs.
308	M	130	90	—	Disch. 30 d.
309	M	136	90	—	Do.
302	M	120	100	+	Died 2 hrs.
303	M	122	100	—	Disch. 30 d.
304	M	122	100	+	Died 4 hrs.
305	M	122	100		Disch. 30 d.
317	M	138	110	+	Died 1 min.
314	M	140	135	+	Died 5 hrs.
315	M	140	135	+	Died 10 hrs.
316	F	144	135	+	Died 1 hr.
318	F	136	135	—	Disch. 30 d.
327	M	110	175	+	Died 30 min.
328	M	120	175	+	Do.
329	M	112	175	+	Died 20 min.
330	F	118	175	+	Died 3 hrs.

[illegible] animals, 110 to 146 gm.
Average weight, 129.20.
[illegible] in amount of [illegible] injected [illegible] to [illegible]

[illegible] Lot No. 10 [illegible]

TABLE 14. *Toxicity of arsenobenzol (Lot No. 271, Philadelphia Dermatological Research Laboratory) when given intravenously to rabbits (ear vein) as a 1 per cent alkaline aqueous solution*

No. of animal.	Sex: M=male, F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
46	M	1.58	30		Disch. 31 d.
45	F	1.73	30		Do.
19	M	2.34	30		Died 16 d.
31	M	2.1	30	−	Disch. 43 d.
20	F	2.1	40	−	Died 46 d.
50	F	1.48	45	−	Disch. 30 d.
51	M	1.55	45	−	Do.
21	M	1.65	50	−	Died 43 d.
22	F	1.65	60	+	Died 5 d.
29	F	2.35	60		Disch. 43 d.
30	M	1.7	60	+	Died 3 d.
35	F	1.6	60	+	Died 6 d.
23	F	1.64	80	−	Died 34 d.
28	F	1.5	80	−	Disch. 43 d.
32	M	1.45	80	−	Died 14 d.
36	F	1.54	80	+	Died 7 d.
24	F	1.62	100	+	Died 10 d.
33	F	1.46	100	+	Died 3 d.
34	F	1.45	100	+	Died 4 d.
37	F	1.5	100	+	Died 3 d.
38	F	1.5	100	+	Died 2 d.
49	M	1.47	125	+	Died 8 d.
25	M	1.57	125	+	Died 2 d.
27	M	1.35	125	+	Died 3 d.
39	F	1.46	125	+	Died 1 hr.
26	F	1.3	150	+	Died 12 hrs.
40	F	1.45	150	+	Died 1 hr.
41	F	1.37	150	+	Died 25 hrs.
42	M	1.84	150	+	Died 6 d.
47	M	1.34	175	+	Died 4 hrs.
48	F	1.45	175	+	Died 3 hrs.
43	F	1.52	175	+	Died 2 d.
44	M	1.23	175	+	Died 2 hrs.

Weight variation, 1.23 to 2.35 kilos.
Average weight, 1.6 kilos.
Variation in amount of fluid injected, 4.8 to 27.6 c. c.

Remarks.—Practically all of the animals which received doses of 100 milligrams or more showed an increase of respiration after the injection. Animal No. 43 developed, after about 24 hours, marked edema of the tissues at the base of the injected ear.

TABLE 15. *Toxicity of arsenobenzol (Lot No. 271, Philadelphia Dermatological Research Laboratory) when given intravenously to rats (tail or leg vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male. F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
24	M	200	20		Disch. 31 d.
23	M	190	20		Died 6 d.
2	M	285	30		Disch. 44 d.
37	M	219	30		Disch. 31 d.
22	F	185	30		Died 24 d.
21	F	182	30		Disch. 39 d.
40	F	200	40		Disch. 31 d.
3	M	280	40		Disch. 44 d.
10	M	250	40	+	Died 1 min.
20	F	160	40		Died 17 d.
1	F	195	50		Disch. 46 d.
6	F	280	50	+	Died 15 hrs.
7	M	280	50	+	Died 1 min.
19	F	160	50		Disch. 39 d.
39	M	200	60		Disch. 31 d.
18	F	150	60		Disch. 39 d.
17	F	130	60		Died 18 d.
16	F	130	60		Disch. 39 d.
4	F	183	70		Disch. 46 d.
8	M	260	70	+	Died 15 hrs.
9	M	250	70	+	Died 1 hr.
15	F	116	70		Disch. 39 d.
26	M	242	80	+	Died 18 hrs.
29	M	212	80	+	Died 10 min.
30	M	202	80		Disch. 33 d.
38	M	190	80		Disch. 31 d.
27	F	172	90	+	Died 12 min.
31	M	190	90	+	Died 10 min.
32	M	185	90	+	Died 3 hrs.
36	M	190	90	+	Died 18 hrs.
5	F	160	100		Disch. 46 d.
11	M	245	100	+	Died 15 hrs.
13	M	210	100	+	Died 1 min.
34	M	157	100	+	Died 18 hrs.
33	M	155	125	+	Do.
35	F	165	125	+	Died 4 min.
12	M	230	125	+	Died 1 min.
14	F	210	125	+	Died 6 min.
28	M	150	150	+	Died 18 hrs.
25	M	150	150	+	Died 3 min.

Weight variation, 116 to 285 gm.
Average weight, 197 gm.
Variation in amount of fluid injected, 0.38 c. c. to 2.87 c. c.

Remark.—Rats Nos. 1 to 14, inclusive, received the drug through one of the tail veins, all others through one of the veins of the hind leg.

None of the rats showed any very decided organic changes at necropsy except rat No. 22 which died of pulmonary disease.

TABLE 16. —*Toxicity of arsenobenzol (Lot No. 271, Philadelphia Dermatological Research Laboratory) when given intravenously to guinea pigs (leg vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
3	M	300	30	−	Disch. 46 d.
1	M	350	30	−	Disch. 42 d.
24	M	320	30	−	Disch. 39 d.
16	F	280	35	−	Disch. 42 d.
4	M	285	50	−	Disch. 46 d.
2	M	310	50	−	Disch. 42 d.
15	M	285	50	−	Do.
21	M	335	50	−	Disch. 39 d.
22	M	330	50	−	Do.
11	M	275	57	+	Died 4 d.
26	M	320	60	+	Do.
27	M	340	60	−	Disch. 37 d.
28	M	320	60	−	Do.
29	M	325	61	+	Died 15 hrs.
31	F	310	70	−	Disch. 31 d.
7	M	275	70	+	Died 3 d.
9	M	280	70	−	Died 15 d.
5	M	300	70	+	Died 20 min.
14	M	295	70	−	Disch. 42 d.
17	M	275	70	+	Died 4 d.
20	M	310	70	+	Died 12 hrs.
23	M	330	70	−	Disch. 39 d.
30	F	325	80	+	Died 5 d.
6	M	275	80	+	Died 4 d.
8	F	300	80	+	Died 7 min.
19	M	300	80	+	Died 1 min.
33	F	320	90	+	Died 1 d.
34	M	310	90	+	Died 7 min.
13		295	90	+	Died 6 d.
12	M	295	90	+	Died 2 min.
32	F	300	100	+	Died 18 min.
10	M	275	100	+	Died 1 hr.
25	M	340	100	+	Died 25 min.
18	M	250	104	+	Died 2 min.

Weight variation, 250 to 350 gm.
Average weight, 304 gm.
Variation in amount of fluid injected, 0.78 to 3.4 c. c.

Remarks.—Nos. 10 and 11 received the drug as a 2 per cent solution.

TABLE 17.—*Toxicity of arsenobenzol (Lot No. 271, Philadelphia Dermatological Research Laboratory) when given intravenously to guinea pigs (leg vein) as a 2 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
43	M.	244	60	+	Died 14 d.
44	F.	240	60	−	Disch. 31 d.
45	M.	240	60	−	Do.
46	F.	240	60	+	Died 10 d.
51	M.	230	70	+	Died 30 min.
52	F.	234	70	−	Died 23 d.
53	F.	236	70	−	Disch. 31 d.
54	M.	234	70	−	Do.
39	F.	228	80	+	Died 30 min.
40	F.	228	80	+	Died 4 d.
41	M.	230	80	+	Died 6 d.
42	F.	230	80	+	Died 4 d.
47	F.	228	90	+	Died 30 min.
48	M.	210	90	+	Do.
49	M.	220	90	+	Died 11 d.
50	F.	220	90	+	Died 6 d.
35	M.	192	100	+	Died 1 min.
36	F.	180	100	+	Died 7 min.
37	M.	198	100	+	Died 25 min.
38	F.	204	100	+	Died 4 d.

Weight variation, 180 to 244 gm.
Average weight, 223 gm.
Variation in amount of fluid injected, 0.72 to 1.02 c. c.

Remarks.—Animal No. 52 probably died of pulmonary disease rather than from the effects of the arsenobenzol.

TABLE 18.—*Toxicity of diarsenol (Lot A 32790-99, Synthetic Drug Co.) when given intravenously to rabbits (ear vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
54	F.	1.47	60	+	Died 9 d.
60	M.	1.82	60	−	Disch. 31 d.
61	F.	1.78	60	−	Do.
62	M.	1.69	60	−	Died 18 d.
100	F.	1.51	80	−	Disch. 33 d.
101	F.	1.53	80	−	Do.
102	F.	1.56	80	−	Died 18 d.
103	F.	1.57	80	−	Died 29 d.
56	F.	1.55	100	+	Died 4 d.
58	M	1.54	100	+	Died 3 d.
59	F.	1.54	100	+	Died 4 d.
63	M.	1.67	100	−	Disch. 31 d.
64	M.	1.29	135	+	Died 4 d.
65	M.	1.38	135	+	Died 15 hrs.
66	F	1.26	135	+	Died 4 d.
67	F.	1.38	135	+	Died 15 hrs.
52	F.	1.54	175	+	Do.
53	M.	1.54	175	+	Do.
55	F.	1.44	175	+	Died 21 hrs.
57	F.	1.67	175	+	Died 15 hrs.

Weight variation, 1.26 to 1.82 kilos.
Average weight, 1.54 kilos.
Variation in the amount of fluid injected, 8.8 to 29.2 c. c.

TABLE 19.—*Toxicity of diarsenol (Lot A 32790–99, Synthetic Drug Co.) when given intravenously to rats (leg vein) as a 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M = male; F = female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
76	F	149	30		Disch. 35 d.
77	M	159	30		Died 17 d.
66	M	180	30	−	Disch. 37 d.
68	M	160	30	−	Do.
65	F	152	50	−	Do.
67	M	154	50	−	Died 24 d.
70	M	120	50	−	Disch. 36 d.
72	M	122	50	−	Died 18 d.
47		164	60	−	Disch. 31 d.
48	F	210	60		Disch. 33 d.
50	M	184	60		Disch. 31 d.
52	M	210	60	−	Died 25 d.
63	M	152	70	−	Disch. 37 d.
69	M	124	70	−	Do.
71	M	120	70	+	Died 2 d.
75	F	137	70	−	Disch. 35 d.
45		177	80	−	Disch. 31 d.
46	M	180	80	+	Died 15 hrs.
49	F	180	80	+	Do
51	M	250	80	+	Do.
143	F	116	80		Disch. 31 d.
144	M	154	80	−	Do.
149	F	160	80	−	Do.
150	F	118	80	−	Do.
56	M	240	83	+	Died 1 min
145	M	118	90	−	Disch. 31 d.
146	M	150	90	−	Do.
151	F	118	90		Died 30 d.
152	F	140	90	−	Disch. 31 d.
41		145	100	+	Died 2 d.
42		150	100		Disch. 31 d.
43	F	170	100	+	Died 15 hrs.
44		170	100	−	Disch. 31 d.
55	M	150	125	+	Died 15 hrs.
58	M	235	125	+	Do.
60	F	115	125	−	Disch. 38 d.
61	F	105	125		Do.
62	M	144	125	+	Died 2 d.
64	M	142	125	+	Do.
73	F	127	125	+	Died 15 hrs.
74	F	117	125	+	Do.
147	M	104	135	−	Disch. 31 d.
148	F	120	135	−	Do.
153	M	130	135		Do.
154	M	108	135	−	Do.
53	M	290	150	+	Died 15 hrs.
54	M	220	150	+	Do
7	M	160	150	+	Do
59	M	138	150	+	Do.

Weight variation, 104 to 290 gm.
Average weight, 156 gm.
Variation in amount of fluid injected, 0.[illegible] to 4.35 c. c.

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Do.
[illegible]	F	[illegible]	[illegible]	[illegible]	Do.
[illegible]	[illegible]	1.4	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	F	[illegible]	[illegible]	[illegible]	Do.
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Do.
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	F	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	F	1.6	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1.4	[illegible]	[illegible]	Do.
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

TABLE 21.—*Toxicity of* [illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
			Mgm.		
[illegible]	M	[illegible]	[illegible]		Died [illegible]
[illegible]	M	[illegible]	[illegible]		Do.
[illegible]	M	[illegible]	[illegible]	—	Died [illegible]
[illegible]	M	[illegible]	80	—	[illegible]
[illegible]	M	[illegible]	[illegible]	—	Do.
[illegible]	M	[illegible]	100	[illegible]	Do.
[illegible]	M	132	[illegible]	—	Do.
[illegible]	M	[illegible]	[illegible]		Do.
[illegible]	M	[illegible]	115	+	[illegible]
[illegible]	M	[illegible]	[illegible]		[illegible]
[illegible]	M	[illegible]	115		[illegible]
[illegible]	M	122	115		[illegible]
[illegible]	M	[illegible]	127	[illegible]	[illegible]
[illegible]	M	[illegible]	125	[illegible]	[illegible]
[illegible]	M	[illegible]	[illegible]		[illegible]
[illegible]	M	[illegible]	[illegible]		[illegible]
[illegible]	F	[illegible]	[illegible]		[illegible]
[illegible]	M	[illegible]	125	+	[illegible]
[illegible]	M	[illegible]	[illegible]		[illegible]
[illegible]	M	[illegible]	[illegible]	+	[illegible]
[illegible]	M	[illegible]	[illegible]		[illegible]
[illegible]	M	[illegible]	150		[illegible]
[illegible]	M	[illegible]	[illegible]	—	Do.
[illegible]	M	[illegible]	150	+	[illegible]
[illegible]	M	[illegible]	150	—	Died 7 hrs
[illegible]	M	[illegible]	[illegible]	+	[illegible]
[illegible]	M	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	M	[illegible]	[illegible]		[illegible]
[illegible]	M	[illegible]	[illegible]		[illegible]

[illegible] to 175 gm.
[illegible] gm.
[illegible]

It will be noticed in the epitome of literature relating to salvarsan that the toxicity of the preparations is denoted by the expressions "Dosis tolerata," "Tolerated dose," "M. L. D.," and "Toxic dose." All of these expressions are indefinite. This is largely because the observation period of the various observers differs. For instance the "M. L. D." may refer either to death within a short time after the administration of the drug or to death from chronic poisoning.

For the sake of clearness and to enable a better comparison of the results obtained in the present investigation the following divisions were made of each series of experiments: (1) The minimal dose which kills 75 per cent or more within 24 hours; (2) the minimal dose which kills 75 per cent or more within 2 weeks; (3) the maximal dose which is tolerated by 75 per cent or more for 24 hours; (4) the maximal dose which is tolerated by 75 per cent or more for 2 weeks.

The results of this investigation are grouped in the above four divisions in tables 22, 23, and 24, thus permitting a ready comparison of the toxicity of the preparations examined.

TABLE 22.—*Showing the comparative toxicity of salvarsan (German), salvarsan (Metz Laboratory), arsenobenzol, and diarsenol when given intravenously to rabbits as a 1 per cent alkaline aqueous solution.*[1]

Preparation	Salvarsan (German).		Salvarsan (Metz	Arsenobenzol.		Diarsenol	
Lot number or date of purchase	1913	A28001 (1917).	A2	257	271	A32790–99.	B72900–11.
Minimal dose which kills 75 per cent or more within 24 hours	175+	175+	175	175+	175+	175	175+
Minimal dose which kills 75 per cent or more within 2 weeks	[2] 100–175	135	100	100	[3] 60	100	135
Maximal dose which is tolerated by 75 per cent or more for 24 hours	175+	175+	135	175+	125	100	175+
Maximal dose which is tolerated by 75 per cent or more for 2 weeks	60	60	60	60	[3] 60	80	100

[1] Doses calculated in milligrams per kilogram of body weight.

[2] Dose probably lies between 100–175 milligrams.

[3] The 80 milligram dose was survived by 75 per cent.

TABLE 23.—*Showing the comparative toxicity of salvarsan (German), salvarsan (Metz Laboratory), arsenobenzol, and diarsenol, when given intravenously to rats as a 1 per cent alkaline aqueous solution.*[1]

Preparation	Salvarsan (German).		Salvarsan (Metz).	Arsenobenzol.		Diarsenol.
Lot number or date of purchase	1913	A28001 (1917).	A2	257	271	A32790–99.
Minimal dose which kills 75 per cent or more within 24 hours	135	135	[2] 100	90	90	150
Minimal dose which kills 75 per cent or more within 2 weeks	135	135	[2] 100	80	90	[3] 125
Maximal dose which is tolerated by 75 per cent or more for 24 hours	70	100	60	70	[4] 60	135
Maximal dose which is tolerated by 75 per cent or more for 2 weeks	70	100	60	70	[4] 60	[3] 135

[1] Doses calculated in milligrams per kilogram of body weight.

[2] The dose of 100 milligrams was regarded as the minimal dose required to kill within 24 hours, or within two weeks, rather than 70 milligrams on account of the fact that the 80 and 90 milligram doses killed less than 75 per cent.

[3] All the animals survived the 135 milligram dose whereas the dose of 125 milligram killed 75 per cent.

[4] The dose of 50 milligrams killed 50 per cent.

TABLE 24.—*Showing the comparative toxicity of salvarsan (German), salvarsan (Metz Laboratory), arsenobenzol, and diarsenol, when given intravenously to rats as a 2 per cent alkaline aqueous solution.*[1]

Preparation	Salvarsan (German).	Salvarsan (Metz).	Arseno-benzol.	Diarsenol
Lot number or date of purchase	A28001 (1917)	A2	257	B72900–11
Minimal dose which kills 75 per cent or more within 24 hours	[2] 90	100	135	150
Minimal dose which kills 75 per cent or more within 2 weeks	[2] 90	70	135	135
Maximal dose which is tolerated by 75 per cent or more for 24 hours	[2] 80	60	80	115
Maximal dose which is tolerated by 75 per cent or more for 2 weeks	[2] 80	60	80	100

[1] Doses calculated in milligrams per kilogram of body weight.

[2] The dose of 90 milligrams killed 75 per cent, whereas the 80-milligram dose killed only 25 per cent.

KEEPING PROPERTIES OF SALVARSAN SOLUTIONS.

The keeping properties of salvarsan solutions were studied, using salvarsan (German, 1917) and arsenobenzol (lot No. 257). One solution of salvarsan (German, 1917) was allowed to stand for from 5 to 6 hours in a glass-stoppered cylinder, the solution only partially filling the cylinder, so that a column of air remained above the fluid. As no precipitate appeared in this solution, it was unnecessary to add sodium hydroxide to clear the solution. A second solution of salvarsan (German, 1917) was examined after it stood for one week in a glass-stoppered cylinder, partially filled. The addition of sodium hydroxide was necessary to clear the solution of the precipitate that had formed. The solution was made alkaline in this way before its administration. In addition arsenobenzol (lot No. 257), which had been kept for one month in a glass stoppered cylinder, partially filled, was examined. This solution was also given as an alkaline solution.

All of the experiments were carried out on rats in the usual manner.

TABLE 25.—*Toxicity of salvarsan, German (1917 sample A28001), after standing 5 to 6 hours under ordinary conditions; given intravenously to rats (leg vein) as a 2 and 1 per cent alkaline aqueous solution.*

No. of animal.	Sex: M=male, F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			mgm.		
431	M	110	50	−	Disch. 30 d.
432	M	110	50		Do.
433	M	110	50	−	Do.
434	M	104	50	−	Do.
427	F	155	60	−	Do.
428	F	120	60	−	Do.
429	M	114	60	−	Do.
430	M	112	60	+	Died 29 hrs.
423	M	112	80	−	Disch. 30 d.
424	M	110	80	−	Do.
425	M	112	80	+	Died 20 hrs.
426	M	110	80	−	Disch. 30 d.

Weight variation, 104 to 155 grams.
Average weight, 115 grams.
Variation in amount of fluid injected, 0.34 to 0.55 c. c.

Remarks.—A portion of the above solution was previously used (immediately after making) for the experiments on rats Nos. 411 to 419, inclusive (see Table 7). No discoloration of the fluid had occurred after standing for six hours, so that it was unnecessary to add alkali for the purpose of clearing the solution. Rats Nos. 431 to 434, inclusive, received the drug as a 1 per cent solution six hours after it was prepared: all others as a 2 per cent solution, about five hours after it was made.

It should be stated further that the above solution was kept in a glass-stoppered cylinder, which was only partially filled, so that a column of air remained above the fluid.

TABLE 26. *Toxicity of salvarsan, German (1917 sample A28001), which was kept under ordinary conditions for one week after being made up as a 1 per cent alkaline aqueous solution. Given intravenously to rats (leg vein) as a 1 per cent solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
206	M	112	50	+	Died 10 d.
207	M	134	50	-	Died 25 d.
208	F	130	50	-	Escaped 25 d.
209	M	140	50	−	Disch. 31 d.
202	M	124	60	+	Died 12 d.
203	F	120	60	-	Died 25 d.
204	F	112	60	+	Died 3 hrs.
205	M	124	60	−	Disch. 31 d.
198	F	110	70	+	Died 15 hrs.
199	F	118	70	+	Do.
200	M	132	70		Disch. 32 d.
201	F	112	70	−	Died 27 d.
196	M	110	100	-	Escaped 25 d.
197	M	112	100	+	Died 15 hrs.

Weight variation, 110 to 140 grams.
Average weight, 121 grams.
Variation in amount of fluid injected, 0.56 to 1.12 c. c.

Remarks.—This solution became turbid upon standing and to redissolve the precipitate which had formed several drops of 15 per cent NaOH were added before injecting the solution.

TABLE 27. *Toxicity of arsenobenzol (lot No. 257, Philadelphia Dermatological Research Laboratory) which was kept under ordinary conditions one month after being made up as a 1 per cent alkaline aqueous solution. Given intravenously to rats (leg vein).*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge
			Mgm.		
222	M	160	50		Disch. 31 d.
223	M	156	50		Do.
225	M	124	50		Do.
235	F	126	50		Do.
234	F	114	60		Died 22 d.
226	F	122	60		Disch. 31 d.
227	F	120	60		Do.
228	M	120	60		Do.
229	F	112	80	+	Died 6 hrs.
230	F	112	80	+	Do.
231	M	110	80		Disch. 31 d.
232	M	110	80	+	Died 6 hrs.
233	M	102	100	+	Died 1 hr.
234	F	102	100	+	Died 3 hrs.

Weight variation, 102 to 160 grams
Average weight, 121 grams
Variation in amount of fluid injected, 0.62 to 1.02 c. c

Remarks.—This solution formed a heavy dark red precipitate upon standing, which was dissolved, before injecting the solution, by the addition of a small amount of 15 per cent NaOH.

It would seem from the above experiments that there was little if any change in the toxicity of these preparations. It can be safely stated that the change in toxicity, if any, after standing for several hours, under the condition of these experiments was so slight that such a possible factor may be disregarded. The above conclusion, however, applies only to alkaline solutions of salvarsan.

SUMMARY AND DISCUSSION OF THE RESULTS OF EXPERIMENTS WITH SALVARSAN PREPARATIONS.

On account of the decided variation in the susceptibility of the test animals employed, the only general conclusion which may be drawn from the present experiments is that there is not a marked variation, if any, in the toxicity of the various preparations of salvarsan examined, and that their toxicity in animals, in a general way, runs parallel with their arsenic content.

However, when we consider the tables more in detail, we find that in Table 22, which gives the summary of the experiments in rabbits, there is a difference of 35 per cent or more in the minimal dose which is required to kill 75 per cent or more of the rabbits within two weeks; also somewhat greater differences in the maximal amounts which are tolerated by 75 per cent or more for the periods of 24 hours and two weeks. It may be concluded further that the 1917 sample of salvarsan (German lot No. A28001) and one sample of diarsenol (lot No. B72900–11) are somewhat less toxic than the other samples examined.

From Table 23, which summarizes the results of the administration of 1 per cent solutions to rats, we may likewise conclude that there is some variation in the toxicity of the various salvarsan preparations. In this group of experiments we again notice that the 1917 sample of salvarsan (German, lot No. A28001) and diarsenol (lot No. A32790–99) are somewhat less toxic than the other samples examined.

From Table 24, which contains the summary of the results of administering the preparations to rats as 2 per cent solutions, we find that diarsenol (lot No. B72900–11) is less toxic than the other samples, and that salvarsan (Metz Laboratory, lot No. A2) is somewhat more toxic than either arsenobenzol (lot No. 257) or diarsenol (lot No. B72900–11).[1]

Individual susceptibility is quite marked in both rabbits and rats. One of the most striking examples of this is to be found in rabbits. In Table 11 it is shown that rabbit 104 survived an intravenous dose of 175 milligrams of arsenobenzol and was discharged at the end of one month; yet 75 per cent of those which received the 100 milligram dose and 50 per cent of those on the 80 milligram dose died within two weeks.

Table 13 shows that there is also considerable individual variation in the susceptibility of rats. All of the rats which were given the 135 milligram dose of arsenobenzol died except one, the rat which was the exception being discharged at the end of one month. Furthermore, 25 per cent of those which received the 60 milligram dose and 50 per cent of those which received the 90 milligram dose died, their deaths occurring in a shorter time than was required for the 135 milligram dose to kill.

The marked individual susceptibility shown above makes it necessary to place more animals than were used in the present investigation on the same dose. In these experiments, as a rule, four or eight animals were placed on the same dose.

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[1] The supply of certain of the samples was limited, and as it was impossible to obtain more material, parallel experiments could not be conducted with all the samples.

While there were no extended observations on the relative susceptibility of pregnant and nonpregnant animals, the impression was gained that pregnant animals were more susceptible than the nonpregnant animals.

Neither rabbits nor rats are entirely satisfactory test animals for determining the toxicity of salvarsan. It would seem, however, that the rat should be given the preference, since it is possible to breed rats under laboratory conditions, and in this way control can be exercised in their selection with reference to such factors as age and weight.

Part of the individual variation in rats may, perhaps, be due to differences in the ages of the animals. To guard against age as a possible factor, in the later experiments rats varying in weight from about 100 to 150 grams were used almost entirely.

By using rats ranging in weight between 100 and 150 grams, and administering the drugs as 2 per cent solutions, the amount of fluid may be limited to about 0.5 to 1 c. c. These amounts may be very conveniently administered to rats or guinea pigs.

In general, it may be said that rabbits tolerate the various salvarsan preparations in intravenous doses of from 60 to 100 milligrams per kilo of body weight for at least two weeks, although we find that certain samples kill in intravenous doses of 60 milligrams per kilo of body weight. Rats tolerate salvarsan preparations in doses of from 60 to 135 milligrams intravenously for a two weeks' period. The results in rats depend somewhat upon whether the drugs are given as 1 or 2 per cent solutions.

The experiments with guinea pigs were confined exclusively to the use of arsenobenzol (lot No. 271). The experiments show that these animals were about as susceptible to this preparation as rats.

It will be noticed that the range between the minimal dose which kills 75 per cent or more within two weeks and the minimal dose which kills 75 per cent or more within 24 hours is very close in rats, whereas it is somewhat wider in rabbits. In other words, salvarsan usually kills rats within a short time; while in rabbits, as a rule, death occurs after a period of several days.

PART II. NEOSALVARSAN AND NEODIARSENOL.

INTRODUCTION.

Neosalvarsan,[1] or "914," which was introduced in 1912 by Ehrlich and Bertheim as an improvement over salvarsan, is a condensation product of salvarsan with sodium formaldehyde sulphoxylate.

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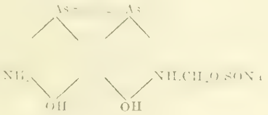
[1] The Federal Trade Commission on March 4, 1918, adopted the name of neoarsphenamine for neosalvarsan and similar products. See Public Health Reports, 1918, vol. 33, p. 540.

Chemically, it is stated to be sodium 3,3′ diamino 4,4′ dihydroxy-arsenobenzol-methanal sulphoxylate. According to Touton (1912) it has the following structural formula:



In addition to the German product neosalvarsan, the compound now appears under the trade names of neodiarsenol (Canada), neokharsivan (England), nov-arsenobenzol (France), and neo-arsaminol (Japan).

Neosalvarsan is an orange yellow powder which is very unstable in air and readily soluble in water, forming a solution which is neutral to litmus. Aqueous solutions are clear, but if a 0.4 per cent saline solution is used, a turbid solution results. The drug is easily oxidized in aqueous solution, its oxidation being hastened by vigorous shaking, or by heating. Its action is similar to that of salvarsan, but it is less toxic. The therapeutic dose, therefore, is larger than for salvarsan. The main advantage in the use of neosalvarsan is that it can be more easily and quickly prepared for medicinal use. Up to the present time the only American substitute for the neosalvarsan of German manufacture is that made by the Synthetic Drug Co., of Toronto, Canada, namely, neodiarsenol.

MANNER OF TESTING NEOSALVARSAN.

Neosalvarsan is tested in the same manner as salvarsan before it is placed upon the market. In this investigation neosalvarsan (German) was compared with neodiarsenol (Synthetic Drug Co.) by determining the results of intravenous injections of the drugs in rabbits and rats. The neosalvarsan preparations were administered to rabbits as 2 per cent aqueous solutions, and to rats as 2 and 5 per cent aqueous solutions. A solution never stood for more than three hours before the last injection was given.

TECHNIQUE EMPLOYED.

The technique, the rate of injection, manner of administration, and the method of antisepsis was similar to that employed for salvarsan. Hæmorrhage in rats was more difficult to control than when salvarsan was used, and in many instances ligation of the vessels was necessary.

SAMPLES OF NEOSALVARSAN EXAMINED.

One sample of neosalvarsan (German) and two samples of neodiarsenol (Synthetic Drug Co.) were examined. The sample of neosalvarsan (German) was obtained in July, 1917, from the American agent of the Farbwerke-Hoechst Co., and bore the lot number A25884, while the samples of neodiarsenol came directly from the Synthetic Drug Co. of Toronto, Canada. Lot number X180-189 was received in June, 1917, while lot number Z5800-11 was received in November, 1917.

COLOR, REACTION, AND SOLUBILITIES OF THE NEOSALVARSAN PREPARATIONS.

All of the samples which were examined were lemon yellow in color, neutral to litmus, and fully soluble in water at ordinary room temperatures.

MANNER OF MAKING THE SOLUTIONS OF NEOSALVARSAN.

Sterile glass-distilled water at room temperature was invariably used in making the solutions. After weighing the neosalvarsan it was put into a glass-stoppered cylinder containing a little less sterile glass-distilled water than was required to make the desired concentration and dissolved by shaking gently. It was then brought to the required concentration by the further addition of sterile distilled water.

LITERATURE RELATING TO THE TOXICITY OF NEOSALVARSAN.

An epitome of the literature relating to the toxicity of neosalvarsan in rabbits is given in Table 28 to facilitate the comparison of the present results with those of previous investigators. No literature on the toxicity of neosalvarsan for rats was available.

TABLE 28.—*Epitome of literature relating to the toxicity of neosalvarsan when given intravenously to rabbits as an aqueous neutral solution.*

Observer.	Where reported.	Preparation.	Dose per kilo.	Remarks.
Castelli	Ztschr. f. Chemotherap. (Orig.) 1912–13, p. 321.	Neosalvarsan (German).	Dosis tolerata 0.3 gm.	Observed 11 days.
Kersten	Centralbl. f. Bakt. 1912, vol. 65, p. 369 (orig.).	...do...	Dosis tolerata 0.2 gm. (average).	Given as a 2 per cent solution; animals observed 6 days.
Marchildon	Deutsch. Med. Wchnschr. 1912, vol. 38, p. 1585.	do	Dosis tolerata less than 0.15 gm	0.2 gm. dose killed all. 0.15 gm. dose killed 67 per cent.
Pearce and Brown	J. Pharmacol. & Exper. Therap. 1917, vol. 9, p. 354.	do...	M. L. D.; 0.150–0.200 gm.	M. L. D. includes death either from acute or chronic poisoning (personal communication).
Spiethoff	Med. Klinik, 1914, vol. 10, p. 581.	do.	Dosis tolerata 0.15 gm.	Observed that rabbits tolerate more neosalvarsan when dissolved in serum than in water.

LENGTH OF TIME ANIMALS WERE UNDER OBSERVATION.

The animals were all under observation for about one month, but for the purpose of this investigation a period of two weeks was chosen as the time limit in the compilation of the following tables. In these tables the minus sign (–) means that the animal has lived for two weeks or longer, while the plus sign (+) means that the animal has died within a two weeks' period.

ARSENIC CONTENT OF NEOSALVARSAN AND NEODIARSENOL.

The arsenic content of the neosalvarsan and neodiarsenol[1] examined was also determined by the method of Ewins (1916). The following table will show the values obtained:

Table 29.

Name of sample.	Manufacturer's number.	Arsenic content.
		Per cent.
Neosalvarsan (German)	A 5884	17.6
Neodiarsenol (Synthetic Drug Co.)	X158	18.6
Do	Z511	15.9

The above table shows that the average arsenic content of the three samples examined is about 17.4 per cent and that the two neodiarsenol samples show a difference in arsenic content of 2.7 per cent.

TABLE 30.—*Toxicity of neosalvarsan, German lot No. A 5884, when given intravenously to rabbits (ear vein) as a 2 per cent aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; –living.	Time of death or discharge.
			Mgm.		
174	M	1.68	60	–	Disch. 30 d.
175	M	1.70	60	+	Died 7 d.
176	F	1.47	60	–	Disch. 30 d.
177	F	1.45	60	–	Do.
158	M	1.60	90	+	Died 5 d.
159	M	1.60	90	–	Died 21 d.
160	M	1.65	90	–	Disch. 31 d.
163	F	1.45	90	–	Do.
161	M	1.55	135	–	Do.
162	F	1.6	135	+	Died 6 d.
164	M	1.75	135	–	Disch. 31 d.
168	F	1.6	135	–	Died 19 d.
165	M	1.57	200	–	Disch. 31 d.
166	F	1.4	200	–	Do.
167	F	1.45	200	+	Died 6 d.
169	F	1.6	200	–	Died 15 d.
170	M	1.6	300	+	Died 5 d.
171	F	1.45	300	+	Died 4 d.
172	M	1.5	300	+	Died 9 d.
173	M	1.51	300	+	Died 4 d.

Weight variation, 1.4 to 1.75 kilos.
Average weight, 1.56 kilos.
Variation in amount of fluid injected, 4.3 to 24 c. c.
Remarks. Rabbit No. 168 died of pulmonary disease; at necropsy No. 175 showed marked inflammation of the peritoneum and considerable excess of peritoneal fluid; death attributed to peritonitis.

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[1] Determination made by Dr. A. S. Seidell, of the Hygienic Laboratory.

Table 31.—*Toxicity of neosalvarsan, German (lot No. A [illegible]), when given intravenously (tail vein) to rats as a 2 and 5 per cent aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
[illegible]	M	110	73	−	Disch. 30 d.
[illegible]	M	142	80	−	Do.
[illegible]	M	112	80	−	Do.
[illegible]	M	160	80	−	Do.
362	F	152	80		Do.
378	M	114	100	−	Do.
379	M	120	100	−	Do.
380	F	104	100	+	Died 4 d.
381	M	112	100	−	Disch. 30 d.
[illegible]	F	110	110	−	Do.
370	F	132	120	−	Do.
[illegible]	M	132	120	+	Died 4 d.
[illegible]	F	115	120	+	Died 20 hrs.
[illegible]	M	110	120	+	Died 20 min.
[illegible]	M	102	140	−	Disch. 30 d.
[illegible]	F	104	175	−	Do.
363	[illegible]	120	175	+	Died 6 d.
364	[illegible]	110	175	+	Died 20 hrs.
365	M	102	175	+	Died 2 d.
366	F	112	250	−	Disch. 30 d.
367	M	112	250	+	Died 4d.
368	M	140	250	+	Died 5 d.
369	M	135	250	+	Died 20 hrs.
371	M	142	[illegible]	+	Died 44 hrs.
[illegible]	F	152	300	+	Died 16 hrs.
376	M	150	300	+	Died 4 d.
377	F	144	300	+	Died 16 hrs.

Weight variation, 102 to 160 grams.
Average weight, 12[illegible] grams.
Variation in amount of fluid injected, 0.4 to 1.5 c. c.

Remarks: Rats Nos. 366 to 369, inclusive, and Nos. 374 to 377, inclusive, received the drug as a 5 per cent solution; all others received the 2 per cent solution.

Table 32.—*Toxicity of neodiarsenol (lot No. X180–180, Synthetic Drug Co.) when given intravenously to rabbits (ear vein) as a 2 per cent aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; −living.	Time of death or discharge.
			Mgm.		
125	F	1.58	90	−	Disch. 31 d.
127	F	1.38	90	−	Do.
128	M	1.42	90	−	Do.
129	M	1.45	90	−	Do.
126	F	1.5	135	+	Died 10 d.
130	F	1.37	135	−	Disch. 31 d.
131	F	1.39	135	+	Died 6 d.
136	F	1.49	135	−	Disch. 31 d.
132	F	1.15	200	−	Do.
133	F	1.46	200	+	Died 2 d.
134	F	1.63	200	+	Died 5 d.
135	M	1.48	200	+	Died 1 d.
157	M	1.4	300	+	Died 16 hrs.

Weight variations, 1.15 to 1.63 kilos.
Average weight, 1.44 kilos.
Variation in amount of fluid injected, 6.2 to 21 c. c.

Table 33.—*Toxicity of neodiarsenol (Lot No. X180 180, Synthetic Drug Co.) when given intravenously to rats (leg vein) as a 2 per cent aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; –living.	Time of death or discharge.
			Mgm.		
214	M	120	80		Disch. 31 d.
215	M	120	80		Do.
240	[illegible]	110	80		Do.
241	F	110	80		Do.
216	F	134	90		Do.
217	F	[illegible]	90		Do.
2[illegible]	M	102	90		Do.
212	M	104	90		Died 20 d.
218	M	[illegible]	100		Disch. 31 d.
219	F	110	100		Do.
245	M	116	100		Do.
244	M	120	100		Died 18 d.
246	F	114	125		Disch. 31 d.
247	F	110	125		Do.
220	M	110	125		Do.
221	F	113	125		Do.
248	F	110	150		Died 18 d.
249	M	108	150		Do.
254	M	108	150	+	Died 6 d.
[illegible]	M	104	150		Disch. 31 d.
[illegible]	[illegible]	108	175		Died 20 d.
251	F	108	175		Disch. 31 d.
257	M	102	175		Do.
255	M	[illegible]	175	+	Died 5 d.
252	M	106	200	+	Do.
253	M	106	200	+	Do.
[illegible]	M	100	200	+	Died 6 d.
[illegible]	M	96	200	-	Disch. 31 d.
[illegible]	M	110	250	+	Died 18 hrs.
[illegible]	F	110	250	+	Do
[illegible]	F	110	250	+	Died 3 d.
296	M	108	250	+	Died 4 d.
297	F	112	300	+	Died 19 hrs.
298	F	110	300	+	Died 17 hrs.
299	F	110	300	+	Do.
300	M	110	300	+	Do.

Weight variation, 96 to 160 grams.
Average weight, 111 grams.
Variation in amount of fluid injected, 0.44 to 1.68 c. c.

Table 34.—*Toxicity of neodiarsenol (Lot No. Z [illegible] 11, Synthetic Drug Co.) when given intravenously to rabbits (ear vein) as a 2 per cent aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in kilos.	Dose per kilo.	Result: +dead; –living.	Time of death or discharge.
			Mgm.		
200	F	1.6	60	+	Died 10 d.
201	[illegible]	1.6	60	+	Died 3 d.
202	F	1.85	60		Disch. 30 d.
203	M	1.7	60		Do.
204	M	1.7	90		Died 20 d.
205	[illegible]	1.5	90	+	Died 10 d.
206	M	1.5	90		Disch. 30 d.
207	[illegible]	1.55	90		[illegible]
208	M	[illegible]	135		Do.
209	F	1.7	135		[illegible]
212	F	1.6	135	+	[illegible]
213	[illegible]	1.55	[illegible]		[illegible]
214	M	1.45	200		Died 20 d.
215	M	1.55	200	+	Died 5 d.
216	M	1.45	200	+	Died 8 d.
217	F	1.6	200	+	Died 3 d.
218	M	1.5	300	+	Died 2 d.
219	F	1.55	300	+	Do.
220	F	1.55	300	+	Do.
221	F	1.5	300	+	Died 15 d.

Weight variation, 1.45 to 2.06 kilos.
Average weight, 1.6 kilos.
Variation in amount of fluid injected, 4.8 to 23.2 c. c.

TABLE 35.—*Toxicity of neodiarsenol (Z5800-11, Synthetic Drug Co.), when given intravenously to rats (leg vein) as a 2 and 5 per cent aqueous solution.*

No. of animal.	Sex: M=male; F=female.	Weight in grams.	Dose per kilo.	Result: +dead; —living.	Time of death or discharge.
			Mgm.		
463	F	120	80		Disch. 30 d.
464	F	120	80	—	Do.
465	M	110	80	—	Do.
466	M	110	80	—	Do.
447	F	110	100	—	Disch. 31 d.
448	M	130	100	—	Do.
449	M	110	100	—	Do.
450	M	120	100	—	Do.
459	M	106	120		Disch. 30 d.
460	M	102	120		Do.
461	M	100	120		Do.
462	M	100	120	—	Do.
468	M	100	150	—	Disch. 26 d.
469	M	110	150	+	Died 2 d.
470	M	110	150	—	Disch. 26 d.
471	M	110	150		Died 20 d.
451	F	104	175		Disch. 31 d.
452	M	100	175	+	Died 2 d.
453	M	106	175	+	Died 4 d.
454	M	108	175	+	Do.
455	F	104	250	+	Died 16 hrs.
456	F	105	250	+	Died 4 d.
457	M	110	250	+	Died 14 hrs.
458	M	110	250	+	Died 3 d.

Weight variation, 100 to 130 grams.
[illegible] grams.
Variation in amount of fluid injected, 0.44 to 0.95 c. c.

Remark.—Rats Nos. 455 to 458, inclusive, received a 5 per cent solution; all others a 2 per cent solution.

In Tables 36 and 37 the results of the various series of experiments with the neosalvarsan preparations examined, are placed in parallel in four divisions, similar to the divisions made for the salvarsan preparations.

TABLE 36.—*Showing the comparative toxicity of neosalvarsan (German) and neodiarsenol when given intravenously to rabbits as a 2 per cent aqueous solution.*[1]

Preparation.	Neosalvarsan (German).	Neodiarsenol.	
Manufacturer's No.	A25884.	X180-9.	Z5800-11.
Minimal dose which kills 75 per cent or more within 24 hours	300+	[2]200+	300+
Minimal dose which kills 75 per cent or more within 2 weeks	300	200	200
Maximal dose which is tolerated by 75 per cent or more for 24 hours	300+	200+	300+
Maximal dose which is tolerated by 75 per cent or more for 2 weeks	200	90	[3]135

[1] Doses calculated in milligrams per kilogram of body weight.

[2] Dose of 300 milligrams given to one animal only, death in 15 hours.

[3] The dose of 60 milligrams killed 50 per cent, whereas the 90-milligram dose killed only 25 per cent of the animals.

TABLE 37.—*Showing the comparative toxicity of neosalvarsan (German) and neodiarsenol when given intravenously to rats as a 2 and 5 per cent aqueous solution.*[1]

Preparation	Neosalvarsan (German).	Neodiarsenol.	
Manufacturer's No.	A25884	X180-9	Z5800-11
Minimal dose which kills 75 per cent or more within 24 hours	300+	300	250+
Minimal dose which kills 75 per cent or more within 2 weeks	120	200	175
Maximal dose which is tolerated by 75 per cent or more for 24 hours	250	200	175
Maximal dose which is tolerated by 75 per cent or more for 2 weeks	100	175	150

Doses calculated in milligrams per kilogram of body weight.

SUMMARY AND DISCUSSION OF THE RESULTS OF THE EXPERIMENTS WITH NEOSALVARSAN PREPARATIONS.

The results of the experiments in which the neosalvarsan preparations were used differed, depending upon whether rabbits or rats were employed as test animals. It is seen from Table 36, which relates to the experiments upon rabbits, that neosalvarsan (German) is distinctly less toxic than either sample of neodiarsenol. In this table only the minimal dose which kills 75 per cent or more within two weeks and the maximal dose which is tolerated by 75 per cent or more for two weeks should be considered, on account of the incompleteness of the figures obtained for the other two dosages. We find that in rabbits it requires 50 per cent more neosalvarsan (German) than either sample of neodiarsenol to kill within two weeks; also, that these animals will tolerate about 50 to 100 per cent more neosalvarsan (German) than neodiarsenol, the difference in percentages depending upon which sample of neodiarsenol is made the basis of comparison.

In Table 37, which relates to the toxicity for rats, the toxicity of neosalvarsan (German) when estimated by the minimal dose which kills 75 per cent or more within two weeks, and the maximal dose which is tolerated by 75 per cent or more for two weeks, is from 50 to 75 per cent greater than that of the neodiarsenol preparations examined.

The results, therefore, are inconclusive as regards the relative toxicity of the neosalvarsan preparations examined.

GENERAL CONCLUSIONS.[1]

1. No marked variation in toxicity was found in either German or American salvarsan, as shown by their intravenous administration to rabbits and rats.

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[1] The work reported in this paper was intended only as a preliminary study, leading to a more detailed examination of a larger number of samples. A consideration of some of the questions which this examination has brought up, is now being made in this laboratory. The later work, as yet incomplete, indicates that the toxicity of the preparations may possibly be changed by modifications of technique.

2. Individual variation in susceptibility to salvarsan is quite marked in both rabbits and rats.

3. The toxicity for rabbits and rats closely parallels the arsenic content of the salvarsan preparations examined.

4. Neosalvarsan preparations were found to be less toxic for rabbits and rats than the salvarsan preparations examined.

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II. ON THE TOXICITY OF EMETINE HYDROCHLORIDE, WITH SPECIAL REFERENCE TO THE COMPARATIVE TOXICITY OF VARIOUS MARKET PREPARATIONS.

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Levy and Rowntree (1916) reported the first fatal case of poisoning due to emetine medication. A man suffering from diarrhea had received daily subcutaneous injections of 1½ grains emetine hydrochloride over a period of 20 days, a total of 29 grains, or 25 milligrams per kilo. Another case, a woman, suffering from pyorrhea, showed severe symptoms after receiving one-half grain daily for 4 days, amounting in all to less than 3 milligrams per kilo. On account of the low dosage, they believed the symptoms resulting in this case to be due largely to an abnormal toxicity of the drug used.

In their review of the literature they present in tabulated form 18 other cases in which undesirable results have followed the use of emetine, though none of these was fatal. The smallest doses leading to toxic symptoms were of 1½ and 4 grains, given subcutaneously as single doses, and ranging from this to 24 grains given over two weeks.

In their conclusions, it is stated that "Patients may differ markedly in their susceptibility to the drug, *and the commercial preparations vary widely in toxicity.*" [Italics ours.] The latter part of this conclusion does not appear to be firmly established, as Levy and Rowntree used only a small number of animals in their toxicity experiments. In considering this point it is obvious that the influence of individual susceptibility to the drug might easily simulate differences in the toxicity of the various preparations tested.

In view of the important bearing which the widely varying toxicity of emetine preparations would have on its therapeutic use, it seemed highly desirable to make a more extensive investigation of the subject.

There is a considerable amount of literature on the historical, chemical, pharmacological, and pharmaceutical aspects of emetine, but on the toxicological and therapeutic side, in which we are particularly interested, the literature is rather scanty. It was not until 1912 that emetine came into rather extensive use, following the favorable reports of Rogers as to its value in the treatment of amebic dysentery. Its use was further extended when Bass and Johns (1915) recommended it in the treatment of pyorrhea alveolaris. Until recently, emetine was apparently considered a relatively nontoxic drug, although the danger of its careless use was appreciated by Vedder (1914). This author warns against the use of large doses of emetine. He reports a few animal experiments, and calls particular attention to the danger of its intravenous administration. He recommends 1 grain subcutaneously as the largest daily dose, this dose not to be continued for more than 10 days.

During the progress of the present investigation there appeared an important report by Johnson and Murphy (1917). They review the most important literature on poisoning in man, and record seven cases of emetine intoxication observed at a base hospital on the Mexican border. Two of these cases ended fatally, one after receiving 25 grains over a period of several weeks, the other after two courses, with a week intervening, amounting in all to 23.5 grains. In both cases muscular weakness was most pronounced, and in one almost constant diarrhea occurred. In one, the necropsy findings were limited to pneumonia and septic bronchitis, in the other there was in addition, fatty degeneration of the heart. In the five nonfatal cases three showed diarrhea and all exhibited motor weakness and nervous disturbances. Evidence of some circulatory disturbances was noted in all. Levy and Rowntree reported diarrhea in their cases. This seems to be one of the most important symptoms of emetine poisoning, and one that might be overlooked when the drug is used in the treatment of diarrheal conditions. Balfour and Pyman (1916) review some of the literature on emetine, particularly in regard to toxicity in man, and present some new clinical observations. They give no experimental data.

The observations on the toxicity of emetine in animals are not extensive. The earlier work gives little reliable information because (1) the number of animals used was small, (2) the doses for the most part were excessive, (3) the doses were, often, not expressed in milligrams per kilo and (4) no attempt was made to establish the minimum lethal dose or the maximum tolerated dose. Some of these errors have been eliminated in the recent work.

Vedder (1914) found that 2.5 milligrams of emetine hydrochloride per kilo, intravenously, killed rabbits in a few seconds; a rabbit which received 20 milligrams per kilo subcutaneously died. White rats were invariably killed by 10 milligrams per kilo, given subcutaneously on two successive days.

Levy and Rowntree (1916) found that 1 to 2 milligrams of the hydrochloride given to dogs subcutaneously daily would kill in from 3 to 15 days. Cats die in 7 to 11 days with 2 to 5 milligram subcutaneous doses daily, and rabbits in 5 to 6 days when given

3 to 4 milligrams per kilo subcutaneously. In regard to intravenous doses they say: "The lethal single intravenous dose is variable (4 to 18 milligrams per kilo in dogs, 6 to 16 milligrams per kilo in cats) and is largely dependent on amount, dilution, and rapidity of injection."

Pellini and Wallace (1916) state that death occurred in cats in a few hours, due to respiratory failure, although a weakened heart action was contributory, when 150 milligrams per kilo were administered subcutaneously; while with smaller doses death occurred in from 18 to 36 hours, due primarily to gastrointestinal changes.

EXPERIMENTAL.

THE GENERAL TOXICITY OF EMETINE HYDROCHLORIDE

It was our original intention to confine this investigation to the comparative toxicity of commercial preparations. The search for the best method of attacking this problem, however, necessitated a considerable number of experiments, and the data thus secured are added as a further contribution to the subject of the general toxicity of emetine hydrochloride. One preparation which the late Mr. Wilbert, of this laboratory, recommended as probably the purest, was used on a large number of animals, including rabbits, white rats, and white mice. A few other market preparations were also used in this preliminary work.

Rabbits. The work on rabbits is summarized in Table 1. When the drug is given intravenously, the result is largely dependent on the concentration of the solution and the rapidity of the injection, as well as on the actual dose given, points emphasized by Levy and Rowntree. Three rabbits received 4 milligrams per kilo (1 c. c. = 10 milligrams) injected in from 15 to 65 seconds, and died in from 15 seconds to 4 minutes. One rabbit received 2.5 milligrams per kilo (1 c. c. = 10 milligrams) injected rapidly. It showed marked symptoms at once, staggering and falling over in great prostration, as did the rabbits just mentioned which died in a few seconds. Within 2 minutes, however, it began to recover and in a short time seemed normal. It was kept under observation for 50 days, and at no time showed any further unfavorable symptoms. Three rabbits received each 5 milligrams per kilo (1 c. c = 2 milligrams) intravenously. In one the injection lasted 5 minutes and 2 minutes later the animal died. In the second the injection time was 7 minutes. This animal died on the sixth day, after showing marked weakness and slight convulsions on the fifth. The third received 3 milligrams during 15 seconds without serious symptoms, and 10 minutes later 2 milligrams were injected in 3 minutes. No immediate symptoms developed, and it remained apparently perfectly normal during 50 days' observation.

The rabbits dying almost at once show fibrillary contractions of the heart, both auricles and ventricles, for several hours, these persisting longest in the right auricle. This feature has been noted by most of the workers on emetine. The rabbit dying on the sixth day showed a slight edema of the lungs, but no other gross lesions. Death may not have been due exclusively to emetine.

Seven rabbits were injected subcutaneously, four of which were fresh animals. One, receiving 25 milligrams per kilo, showed diarrhea on the following day and died after 40 hours. The lungs showed edema and small hemorrhages, the intestinal tract slight early inflammatory changes, and the kidneys an acute parenchymatous nephritis (confirmed by microscopic study). Two received 20 milligrams per kilo, one of which died on the fifth day, the other on the seventh day. Diarrhea was noted in one. One showed no definite lesions at necropsy; the other, inflammatory areas throughout the intestinal tract, and some degenerative changes in the liver and kidneys. One received 15 milligrams per kilo and remained normal except for a slight weight loss. After 50 days it was given a second injection of 15 milligrams per kilo and never showed any symptoms of the drug, finally dying from distemper 120 days after the last injection. Two rabbits, recovering from intravenous injections after 50 days, were given 20 and 25 milligrams per kilo and died in 60 and 42 hours, respectively. The chief symptoms were weakness and diarrhea. At necropsy, the rabbit dying after 42 hours showed fine hemorrhages in the lungs and congestion of the intestinal mucosa. Microscopic examination of the liver and kidneys stained with osmic acid and hematoxylin and eosin showed slight fatty changes in the former and a well marked parenchymatous tubulo-nephritis, with considerable fatty change, in the latter. The other animal showed fatty and parenchymatous degeneration of the liver and a slight inflammatory condition of the intestine.

From these few experiments the intravenous method seems quite hazardous, and we quite agree with Vedder, Levy and Rowntree, and others that its use is seldom, if ever, justifiable in man. On account of this wide variation in results, we hesitate to express an opinion as to the minimum lethal dose intravenously. Subcutaneously, 25 milligrams per kilo seem to be an acutely fatal dose in rabbits; 20 milligrams a fatal dose, but causing death less rapidly; while 15 milligrams are probably near the maximum tolerated dose.

Rats.—We worked also with white rats, in order to determine their suitability for the comparative study of the various market preparations. The summarized results of this work are shown in Tables II and III. The points we wished to determine were, the maximum tolerated dose, whether giving repeated doses would materially increase the value of the experiment, the symptoms produced and the pathological changes. All doses were given subcutaneously. In all, 54 rats were used, 37 with single doses, 17 with repeated doses. With 40 and 50 milligram doses per kilo, there was no great difference in the time of death, none of the animals living 24 hours. With 30 milligrams per kilo, the average time of death is increased to 30 hours; and, with 25 milligrams per kilo, to 44 hours. Twenty milligrams per kilo seems to be the minimum lethal dose, as none of these animals recovered. There is a wide range in the time of death, from 1 to 18 days. The greater number of the animals, however, die acutely. When the dose is reduced to 15 milligrams per kilogram, none of the animals die within the acute period; but, on the other hand, none recovered. In two, complications were found which probably hastened death. With a larger series there would probably be some recoveries. We are probably correct in placing the minimal lethal dose at between 20 and 15 milligrams per kilo. With 10 to 12 milligrams per kilo, half the animals lived 96 days, and were in good condition at the end of the time. Two died on the seventieth and eighty-first days, probably from causes other than emetine. One, dying on the fourth day, had a local infection on the neck, unnoticed until after injection, and should not be counted. These smaller doses are probably near the maximum tolerated dose of the drug, and it will be seen that there is not a very large margin between this and the minimum lethal dose.

The small series receiving repeated sublethal doses (Table III) shows that 10 milligrams per kilo, repeated once, were always fatal (see Vedder), the time of death varying from 40 to 128 hours. When this dose was repeated twice, the average time of death was 47 hours; a result quite comparable to 25 milligrams given as a single dose. Of five rats receiving 5 milligrams per kilo doses, one died on the eleventh day, after 7 doses; one on the sixth day, after 5 doses; and the other three in 4 to 6 days, after 4 doses. This suggests a cumulative action, on account of incomplete elimination of the poison, or that death results from a summation of injuries. Much of the experimental work done with emetine has been with repeated doses, probably because it is so used in medicine. However, as the chief object of our work is the comparative toxicity of various preparations, we see no reason why the single injection method is not fully as accurate as the fractional injection. The work with mice, mentioned below, also tends to show this.

By far the most marked symptom in rats is diarrhea. This is accompanied by loss of weight and of appetite. In the very acute cases cramps were occasionally noted, and a general increase in the reflex irritability, with some paresis of the hind legs. In a number of animals, which died, no particular symptoms were noted, other than loss of weight which was invariably present.

At necropsy, changes in the liver were nearly always present, even when death occurred in less than 24 hours. This varied, from a slight paleness to definite, gross, fatty, and parenchymatous changes. The intestine sometimes seemed nearly normal in the very acute cases, but as a rule there were definite inflammatory changes. The kidney seems to be involved later, though occasionally as early as 48 hours. The pathologic findings in the rats were based on the gross appearances.

Mice.—As the work progressed, mice were found much more suitable than rats, as during the summer diarrhea appeared quite often among the normal rats without discoverable causes, while there was little trouble in keeping our stock mice in good condition. It might be well at this time to call attention to the undesirability of using mice a second time in experimental work of this character. At the time we began this work there was on hand a large number of mice which had received small doses of phenol or glycerin and which had recovered and were apparently healthy. It was suggested that they might be suitable for this work. Table IV shows the comparative results between 35 fresh mice and 31 used mice. Study of this table shows that, with acutely fatal doses, the used mice die in a slightly shorter average time. With smaller doses some used animals die within the acute period, although most of them die later on, in from several days to weeks. On the other hand, most of the fresh mice are living and well 30 days after sublethal doses. This emphasizes the fact that if it ever be permissible to use animals a second time in toxicity work it should be only for rapidly fatal doses, and even then the results should be checked against normal animals. Fifty of these used mice were chloroformed and necropsied. About 25 per cent showed definite gross lesions, most often in the kidney. Ten fresh mice were treated likewise, and none showed definite gross lesions. None but fresh, healthy appearing mice were used in our experiments, with the exception of a few with higher doses, which are indicated in the tables. It seems that wrong conclusions might easily be reached if subnormal or used animals are not excluded.

From the small number of mice used in the preliminary work three or more with each dosage, 50 to 60 milligrams per kilo seem to be the minimal dose causing death acutely, while 25 seems to be near the maximum tolerated dose. As will be seen later, when large numbers of mice are used and the time of observation increased the results obtained differ considerably, even with the same preparation of the drug. When repeated sublethal doses are given (see Table V) the time of death is delayed, and a larger total dose required. When 5 milligrams are given daily, as many as 16 doses were tolerated fairly well. Two such animals were given a 60 milligram per kilo dose; both died acutely, showing that no tolerance had been acquired. After trying several market preparations on nearly 100 mice, using 100 milligrams and 60 milligrams per kilo as single doses, and 10 and 20 milligrams per kilo as repeated doses (tabulated report omitted), it was found that as a rule 100 milligrams killed quite acutely, 60 milligrams acutely, and that repeated doses of 10 and 20 milligrams, amounting in all to 60, killed with regularity, but required a much longer time. It seemed that the single doses gave results quite as comparable as the repeated doses. Hence several market preparations were tried on series of 12 mice each, with 60, 50, 40, and 30 milligrams per kilo doses, i. e., three mice to each dosage. It soon became evident, from the variations encountered, that a larger number of animals should be used with each dosage.

The symptoms produced in mice are not very characteristic. In acute cases there is sometimes an increased reflex irritability and some paresis. The hair is roughened and the animal appears greatly prostrated. Often they remain in a semicomatose condition some hours before dying. In those dying subacutely diarrhea is often, but not always, present. Those dying late show a gradual loss in weight and evidence of malnutrition. Diarrhea may persist a long time in such cases.

Practically all the mice were necropsied and the gross findings recorded. Twenty were studied microscopically. The earliest gross change seems to be in the liver, as is the case in rats. In fact, the changes in mice and rats are similar, but they were more extensively and carefully studied in mice. The liver appears slightly increased in size, and the gross appearance suggests fatty changes. Very marked changes were noted in less than 24 hours and from that to several days. Stained with Scarlet R. or with osmic acid, histologic examination showed marked fatty infiltration and degeneration. Stained with hematoxylin and eosin, a considerable parenchymatous degeneration was also evident. Occaasionally the kidneys and intestine also showed changes within 24 hours, though taking all cases into consideration the intestine seemed to be involved slightly later than the liver and the kidney last. Only rarely were changes found in the lungs and then as scattered ecchymoses. There was almost never any local reaction at the site of injection, nor were there other important changes. In other words, the parenchymatous organs were chiefly involved. The kidneys, microscopically, showed acute parenchymatous degeneration, chiefly of the proximal convoluted tubules, plus fatty degeneration.

Mice dying after several days frequently show little gross change in the liver, but often a more or less severe enteritis; in fact, there was practically always some enteritis, except in the very acute cases. Often this was haemorrhagic in type. No distinct ulcers or perforations were ever noted. Microscopically, the mucosa showed marked degeneration, being largely destroyed in certain areas.

Mice observed over 20 days, whose weight remained stationary or increased, and which appeared normal, usually showed no gross changes; but when changes were present, the kidney was most often involved. Mice showing a loss in weight, and appearing generally subnormal at this time, usually showed gross changes in the kidney, rarely in the liver and intestine. Studied microscopically, some of these livers showed parenchymatous changes, and the kidneys parenchymatous and diffuse nephritis.

To summarize: The liver, intestines, and kidney are the organs chiefly attacked in mice, and apparently in the order named. Probably the liver often regains a practically normal condition, if the animal survives the acute action of the poison. After the very acute stage, the intestine is nearly always involved; and the kidney is nearly always involved, even when the mice appear only subnormal.

This pathology is slightly different from that described by Podwyssotzki (1879), Lowin (1902), Levy and Rowntree (1916), and others, in that early definite fatty changes were quite marked in our animals. This difference may, however, be due to the species of animals used.

THE COMPARATIVE TOXICITY OF TWELVE COMMERCIAL PREPARATIONS[1] OF EMETINE HYDROCHLORIDE.

Mice were used exclusively as the preliminary work had shown them to be the most suitable and available animals for this purpose. The drug was always administered subcutaneously because (1) it is the most convenient method, (2) it is the method commonly used in man for emetine medication, and (3) it is less apt to lead to widely varying results than the intravenous method, for reasons mentioned in the preliminary part of this paper. The subcutaneous method, generally speaking, has the disadvantage of introducing a complicating factor as to rate of absorption. This disadvantage is largely eliminated as emetine produces little or no local irritation of the subcutaneous tissue and seems to be readily absorbed. The diet consisted of oatmeal and milk which, in the experience of this laboratory, had been found to be the most satisfactory. It was given for some time preceding the experiment as well as during the entire period of observation.

The results have been briefly summarized in Table VI, which shows the length of time required to kill, according to the preparation and dosage used, and the number of mice surviving any given experiment. The largest number of mice used with any one preparation was 120, the smallest 60. Throughout the work, groups of 10 animals were given the same dosage of each preparation at the same time, and all animals surviving the acute effect of the drug were observed, 30 days or longer. Healthy, nearly full-grown mice were selected from the laboratory stock. Ordinary asepsis was observed, the injections being made with syringes graduated to hundredths of a cubic centimeter and fitted with a very fine needle, which practically eliminated the danger of leakage through the needle puncture. It should be stated that, with very few exceptions, those animals living at the end of the period of observation were apparently normal, with no loss of body weight. Many of the surviving mice were chloroformed and necropsied. Those showing a loss in weight and a generally subnormal appearance usually showed gross lesions, most often of the kidney, an observation already mentioned.

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[1] Between May 20 and July 20, 1916, 12 different preparations of emetine hydrochloride, from 8 different firms were purchased on the open market.

At first the intention was to find the minimal lethal dose for all preparations, as well as the maximum tolerated dose, and to use these data for comparative purposes. As the work progressed, it became evident that it was difficult to establish these points, unless arbitrary standards were fixed, as to length of time in which the minimal lethal dose should kill, and the minimum length of time during which all the animals of a group should live, to consider the dose a tolerated one.

We finally found it practically impossible to get the maximal tolerated dose to a point where none of the animals would die during a period of 30 days, unless a very large number of mice were used with very gradually decreasing doses; this would be a study of susceptibility rather than of comparative toxicity. It soon becomes evident, on study of the table, that if the preparations be arranged as to toxicity with any two or three dosages, the order is far from constant; in fact, it seems impossible to range the order of toxicity for the following reasons: If the number of days elapsing before the last animal dies is considered an index, it may be far from correct, as 8 mice may die relatively acutely and the other two live a long time or recover completely; if, on the other hand, the average time of death is considered, it may be far too long for the same reason. In either case an occasional tolerant animal spoils the ratio. From the lower-dosage standpoint, the reverse is true; the occasionally highly susceptible animal makes average values unreliable. If we arbitrarily fix the maximum time within which all animals should die, we may get far too high a dosage, for similar reasons. In other words, the striking thing about the whole experiment is the very marked individual variation in susceptibility and tolerance.

That this variation in individual susceptibility is an extremely important factor in toxicity work is emphasized by the work of Schamberg, Kolmer, and Raiziss, 1915. These authors, in perhaps the most extensive investigation yet made on the toxicity of mercury found great difficulty in arriving at tolerated doses, on account of inconsistencies. They used 250 rats with mercurials, and, despite all precautions, repetition of doses, etc., got occasionally inexplicable findings. They emphasize the fact that mathematically precise results can not be expected, on account of individual variations.

We not only find individual variation in the same lot of 10 animals, for example, but what might be termed *group variation*. It will be seen that, while in general with any given preparation the results correspond with the dosage, there are several instances where this fails to hold. As several dosages were given from the same solutions (it having been determined that the drug remains perfectly stable in solution for a considerable time when made up with sterile distilled water and kept in ice box in well stoppered bottles), this can not be accounted for by variations in the dosage.

While the general trend of the experiment is probably sufficiently shown by the chart, a brief discussion of what seem to us the most important points may not be out of place.

The 60 milligrams per kilo dose was used first, as from the preliminary work it seemed near the minimum lethal dose. Six out of the 12 preparations killed all animals fairly acutely, with the exception of No. 7, where one lived 26 days; the average time of death varied from 43 to 64 hours, with the exception of No. 7, where it was 99 hours. With the other 6 preparations from 1 to 3 animals lived throughout the experiment. The greatest difference seems to be between No. 2 and Nos. 11 or 12. No. 2 killed animals in an average time of 48 hours, 1 died on the sixteenth day and 3 survived; of these 3, 2 showed definite kidney lesions. One out of the ten apparently had suffered in no way from the drug. No. 12 gave a quite uniform result, killing every mouse within 28 to 60 hours, the average time being 47 hours. It is impossible to express any percentage of difference between the two. It will be noted that, when 70 milligrams per kilo were used No. 2 killed nine in an average of 36 hours, while one lived 128 hours; with 80 milligrams per kilo, all were killed in an average of 30 hours.

With 50 milligrams per kilo the mortality was 100 per cent in only two groups. With No. 3 all mice were dead within the fourth day. This is the most marked group variation of all. It will be noted that, with the higher dose, two mice had recovered. Moreover, No. 12, which had seemed most toxic with the next higher dose, with this doasge seemed least toxic.

When higher doses are given, the discrepancies are not quite so marked. A 70 milligrams per kilo dose was used with all preparations but No. 12. The mortality was 100 per cent in all but two groups, where two animals in each lived throughout the experiment. With the exception of these two groups the average time of death was from 26 to 61 hours. With 80 milligrams per kilo doses seven preparations were used, with the resulting death of every animal in an average time of from 27 to 38 hours.

Taking into consideration all the doses causing death acutely, in from three to four days as a maximum, the greatest variation in dosage seems to be from 60 to 80 milligrams per kilo.

The maximum tolerated doses have not been finally determined, and from the data at hand it is difficult to state whether or not any greater variations are shown than with higher doses. At any rate the differences here do not help to show any particularly high toxicity connected with any given preparation.

It is noticeable that, no matter what the dosage, most of the mice that die, die relatively acutely, as shown by the following figures for 880 mice injected with various doses of emetine hydrochloride:

Died during the first day after the injection	86
Died during the second day after the injection	214
Died during the third day after the injection	96
Died during the fourth day after the injection	25
Died during the fifth day after the injection	40
Died during the sixth day after the injection	17
Died during the seventh day after the injection	26
Died during the second week after the injection	13
Died during the third week after the injection	27
Died during the fourth week after the injection	8
Survived at least 30 days	328

By far the greatest portion of the deaths occur during the first three days, while the total number dying after the seventh day is less than 10 per cent of the total number dying altogether.

It is impossible to say how many of this small number dying after the first week owe their death exclusively to emetine, as the lesions produced are not pathognomonic. However, inasmuch as controls die very rarely, it seems probable that the emetine was contributory as a cause of death in most of them.

The most probable explanation for the fairly uniform toxicity of the preparations used in this work seemed to be either a common source, or the use of a process yielding a uniform product. Letters, to clear up these points were accordingly written to the different firms, which were very courteous in replying promptly as fully as possible. Their replies show that most of the firms manufacture their own product, though by somewhat different processes. Some firms buy their entire supply from others and simply market it.

Preparations No. 6 and No. 10 are in all probability the same product. It will be noted that the results check quite closely on those two. Both the Rio and Carthagena roots are used, some firms using one only, some both varieties.

Judging from those letters, as well as from the literature, the greatest difficulty in the process is the complete separation of emetine and cephaëline. While the latter is somewhat more toxic (see Lewin, 1902), a fairly high per cent would need to be present to affect materially the toxicity.

It is probable that the best firms use the necessary precaution and make the separation practically complete, and that none of the preparations on the market contain any considerable amount of this alkaloid. Had sufficient variations in toxicity been found, it was our intention to investigate cephaëline alone, and in combination with emetine. However, in view of our results, this hardly seems necessary at the present time.

TABLE I.—*Toxicity of emetine hydrochloride on rabbits.*

PREPARATION I.

Experiment No.	Weight	Route	Mgs./kg.	Concentration	Duration of injection	Symptoms	Result	Remarks on necropsy, etc.
1	1,600	S.C.	20	1 c.c.=10 mg.		No definite symptoms	Died on fifth day . .	Intestinal tract throughout shows inflammatory areas, a moderate nephritis, and some degeneration changes in liver.
2	1,600	S.C.	15	do.	. .	No definite symptoms, but gradual loss in weight	After 50 days in fair condition, weight, 1,550 most marked change	Used again under No. 14.
3	2,530	I.V.	4	do.	15 seconds. .	Staggers and rolls over, collapse	Died in 15 seconds.	Right ventricle enormously distended, left contracted; no other changes.
4	2,360	I.V.	4	do.	45 seconds. .	do.	Died in 4 minutes	No gross changes; fibrillary contraction of auricle for several hours.
5	2,330	I.V.	4	1 c.c.=2 mg. .	do.	do.	Died in 30 seconds	No definite changes (fibrillary contraction for hour).
6	1,900	I.V.	5	do.	7 minutes	On fifth day weakness, tremors, slight convulsions	Died on sixth day. .	Pulmonary edema, ht. marked dilations; no changes in intestines.
7	2,750	I.V.	2.5	1 c.c.=10 mg.	5 seconds. .	At end of injection staggers but recovers in 2 minutes, never any marked symptoms later	After 50 days seems entirely normal	Used again in experiment 12.
8	2,200	I.V.	5	1 c.c.=2 mg.	5 minutes.	[illegible] and collapse.	Dies 2 minutes after injection	Heart beats for a long time afterwards.
9	2,150	I.V.	5	do.	3 mg. in 15 seconds, 2 mg. in 3 minutes, 10 minutes later	No immediate symptom.	After 50 days seems entirely normal	Used in experiment 13.
10	1,750	S.C.	20	1 c.c.=10 mg.		Diarrhea on following day. .	Dies in 40 hours	Lungs, edema, and small hemorrhages, nephritis, intestines severe congestion, and early inflammatory changes.
11	[illegible]	S.C.	20	do.		Diarrhea complicated by terminal convulsion	Dies on seventh day	No very definite changes; cause of death not found.
12	2,500	S.C.	25	do.			Dies in 42 hours	Lungs, fine hemorrhages, marked congestion only.
13	2,000	S.C.	20	do.		Diarrhea and weight loss, weakness	Dies in 60 hours.	Liver fatty and parenchymatous; intestines slight inflammation.
14	1,550	S.C.	15	do.		None.	Death in 62 days. .	Distemper.

TABLE II.—*Toxicity of Emetine Hydrochloride on White Rats.*

PREPARATION 1.—SINGLE DOSES.

No.	Sex.	Weight.	Dose, mg./kg.	Duration of experiment.	Result.	Remarks.
[illegible]	M.	150	[illegible]	[illegible]	Died	
[illegible]	M.	[illegible]	50	[illegible]	do.	
28	F.	[illegible]	43	[illegible]	do.	
[illegible]	M.	[illegible]	40	[illegible]	do.	
[illegible]	M	[illegible]	40	[illegible]	do.	
[illegible]	M.	140	40	[illegible]	do.	
[illegible]	M.	[illegible]	40	[illegible]	do.	
[illegible]	M.	152	40	[illegible]	do.	
[illegible]	M	115	30	[illegible]	do.	
[illegible]	F.	110	[illegible]	[illegible]	do.	
[illegible]	M.	150	30	[illegible]	do.	
[illegible]	M.	[illegible]	[illegible]	[illegible]	do.	
33	M.	[illegible]	[illegible]	[illegible]	do.	
[illegible]	F.	[illegible]	25	[illegible]	do.	
[illegible]	F.	115	25	[illegible]	do.	
[illegible]	M.	[illegible]	25	[illegible]	do.	
[illegible]	F.	[illegible]	[illegible]	[illegible]	do.	
[illegible]	F.	245	[illegible]	[illegible]	do.	
[illegible]	M.	[illegible]	20	[illegible]	do.	
[illegible]	F.	[illegible]	[illegible]	[illegible]	do.	
39	M.	219	20	[illegible]	do.	
41	M.	120	20	[illegible]	do.	
[illegible]	F.	105	[illegible]	[illegible]	do.	
[illegible]	F.	87	20	[illegible]	do.	
[illegible]	F.	[illegible]	20	[illegible]	do.	
40	M.	115	15	[illegible]	do.	Lung disease.
[illegible]	M.	182	15	[illegible]	do.	
[illegible]	M.	[illegible]	15	[illegible]	do.	
[illegible]	M.	[illegible]	[illegible]	[illegible]	do.	
[illegible]	M.	133	15	[illegible]	do.	
[illegible]	M.	[illegible]	15	[illegible]	do.	
[illegible]	M.	[illegible]	12	[illegible]	do.	Complications.
[illegible]	M.	177	12	[illegible]	Living.	
[illegible]	M.	182	12	[illegible]	Died.	Decomposition.
[illegible]	M.	201	10	[illegible]	Living.	
[illegible]	M.	[illegible]	10	[illegible]	do.	
57	M.	[illegible]	10	[illegible]	Died.	Complications.

TABLE III.—*Toxicity of Emetine Hydrochloride on White Rats.*

PREPARATION 1. REPEATED DOSES.

No.	Sex.	Weight.	Dose, mg./kg.	Number doses.	Total.	Result.
13	M.	[illegible]	20	2	40	Died, 36 hours.
14	M.	122	20	2	40	Died, 37 hours.
41	M.	245	5	7	35	Died, 11 days.
16	M.	152	10	3	30	Died, 47 hours.
17	M.	152	10	3	30	Died, 54 hours.
20	M.	155	10	3	30	Died, 47 hours.
6	F.	105	5	5	25	Died, 144 hours.
15	M.	250	5	[illegible]	20	Died, 120 hours.
9	F.	110	[illegible]	[illegible]	20	Died, 144 hours.
21	M.	137	5	4	[illegible]	Died, 108 hours.
15	M.	175	10	2	[illegible]	Died, 40 hours.
22	M.	150	10	2	20	Died, 42 hours.
23	F.	150	10	2	20	Died, 126 hours.
24	M.	90	10	2	20	Died, 89 hours.
25	M.	95	10	2	20	Died, 128 hours.
26	M	110	10	2	20	Died, 59 hours.
27	M	103	10	2	20	Died, 91 hours.

Table IV.—*Toxicity of emetine hydrochloride on white mice.*

PREPARATION 1. SINGLE DOSES.

FRESH MICE.

Dose, mgs./kg. S. C. route.	Number mice used.	Died acutely.				Subacute.		Chronic.		Lived.		Remarks.
		No.	Min.	Max.	Av.	No.	Av.	No.	Av.	No.	Time	
			Hours.	*Hours.*	*Hrs.*		*Hrs.*		*Days.*		*Days.*	
100	2	2	22	28	25	0	....	0	.....	0	.....	
60	10	8	26	88	48	2	132	0		0	.....	
50	3	3	68	102	87	0	.....	0	.....	0	...	
40	3	0	.....	......	.....	0	.....	0	......	3	18	2 below normal.
30	3	0	......	......	......	1	120	0		2	18	1 below normal.
25	6	0	......	...	...	0	.....	1	12	5	37	In good condition.
20	6	0	......	..	...	0	....	1	33	5	37	Do.

USED MICE.

Dose, mgs./kg. S. C. route.	Number mice used.	Died acutely.				Subacute.		Chronic.		Lived.		Remarks.
		No.	Min.	Max.	Av.	No.	Av.	No.	Av.	No.	Time	
100	3	3	4	28	19	0	......	0	......	0	......	
85	3	3	22	28	24	0	....	0	.....	0	...	
80	4	4	10	26	18	0	......	0	......	0	......	
60	4	4	18	61	45	0	....	0	.....	0	.....	
50	5	3	64	70	66	0	.....	2	9–10	0	......	
40	3	2	90	96	93	0	.....	0	......	1	27	Seems normal.
30	3	0	....	....	....	0	......	3	8–22	0	......	
25	6	1	...	.....	72	0	......	4	20–36	1	60	Do.

Table V.—*Toxicity of emetine hydrochloride on white mice.*

PREPARATION 1—REPEATED DOSES.

No.	Sex.	Weight.	Mgs./kg.	Number of doses.	Mgs./kg., total amount.	Result.
10	M.	18.5	20	4	80	Died, 116 hours.
11	M.	19.5	20	4	80	Died, 117 hours.
31	M.	19.0	20	4	80	Died, 100 hours.
32	F.	17.2	20	4	80	Died, 87 hours.
33	M.	16.0	20	4	80	Died, 86 hours.
34	F.	19.2	10	7	70	Died, 8 days.
35	F.	16.5	10	7	70	Do.
36	F.	15.0	10	9	90	Died, 12 days.
16	....	21.0	5	16	80	Died, 24 days.
17	....	19.5	5	16	80	L. and W., 27 days; 60 mgs./kg. kill in 5 hours.
18	....	18.0	5	16	80	L. and W., 27 days; 60 mgs./kg. kill in 3 days.

TABLE VI.—*Toxicity on white mice of 12 commercial preparations of emetine hydrochloride.*

	Dosage, Mg./Kg	Number of mice dying during the intervals specified below.											Number days observed.	Number alive at end of experiment.
		12–24 hours.	24–36 hours.	36–48 hours.	3d day.	4th day.	5th day	6th day.	7th day.	2d week.	3d week.	4th week.		
No. 1....	[1] 80	7		1	2								3	0
	[1] 70	1	5	2	1		1						5	0
	60		2	4	1	1		2					6	0
	50		2		5		1	1			1		22	0
	40				1		2						33	7
	30				1	1					1	1	33	6
	25		2										31	8
	20		1						1	2	1	1	30	4
	15				1								30	9
	12												30	10
No. 2....	90	4	1	3		2							4	0
	[1] 80	6	2		1	1							4	0
	70	2	4	1	2			1					6	0
	60	1	1	3			1			1			34	3
	50	2	1	1	2		1		1				33	2
	40	2		2			2		1		1	1	33	1
	30		3					1	1				31	5
	20			1							1		30	8
	15												30	10
No. 3....	70	3	5	1	1								3	0
	60	1		1	4	1			1				36	2
	50		2	3	3	2							4	0
	40		2	1	1	1		1					33	4
	30		1										31	9
	20	1											30	9
	15											1	30	9
No. 4....	70	3	4	1	2								3	0
	60			5	1		2	1					34	1
	50	1			2		4	1	1				33	1
	40	1	1	5	1		1						33	1
	30		1		2	1			1				31	5
	20	1									1		30	8
	15												30	10
No. 5....	80	2	7		1								3	0
	70		2	5		1							36	2
	60	2	1		1	2			3				34	1
	50		1	1	4			2					33	2
	40	1		1	1	1							31	6
	30											1	30	9
	20											1	30	9
No. 6....	[1] 80	5		4	1								3	0
	[1] 70	1	6			1	1	1					6	0
	60	4	1	1	2		1	1					6	0
	50	1			3				3		1		36	2
	40				2		1		2		2		33	3
	30		2	1									33	7
	25			1							2		31	7
	20							1		1			30	8
No. 7....	70	3	4	2	1								3	0
	60		6		3						1		26	0
	50		1	2	2		1					1	36	3
	40			4	1		1		1			1	33	2
	30		3				2				1	2	31	2
	20										1		30	9
No. 8....	[1] 80	5	1		4								3	0
	70	1	3	1	2		2	1					6	0
	60	1	2	1	2	1				1			34	2
	50		1		3		2				2		33	2
	40		1	1		1			2		2		33	3
	30	1									1		31	8
	20											1	30	9
No. 9....	80	5	3	1	1								3	0
	70		2	3	2				1				36	2
	60	1	1	1	1	1	3				1		34	1
	50	1	1	4			1						33	3
	40		2	2	2								31	4
	30									1			30	9
	20								1	1	1	1	30	6
No. 10...	80	1	4	3	1	1							4	0
	[1] 70	1	2	5	1				1				7	0
	60	1	1	4	2		2						5	0
	50	2	1	2	2	1			1				36	1

[1] This lot consisted of apparently healthy mice each of which had received several weeks previously a small dose of emetine hdyrochloride.

Table VI.—*Toxicity on white mice of 12 commercial preparations of emetine hydrochloride*—Continued.

	Dosage, Mg./Kg.	Number of mice dying during the intervals specified below.											Number days observed.	Number alive at end of experiment.
		12-24 hours.	24-36 hours.	36-48 hours.	3d day.	4th day.	5th day	6th day.	7th day.	2d week.	3d week.	4th week.		
No. 10	40	...	1	...	2	3	1	...	...	...	1	...	33	2
	30	...	...	...	...	...	1	...	...	...	...	2	33	7
	25	...	...	...	1	...	1	...	...	...	2	...	31	6
	20	...	...	...	...	...	1	...	...	1	...	...	30	8
No. 11...	70	2	6	...	2	...	...	...	...	...	...	...	3	0
	60	3	2	2	1	1	1	...	...	...	...	...	5	0
	50	2	3	2	1	...	...	1	...	...	...	...	36	1
	40	3	...	2	1	...	1	...	1	...	...	...	33	2
	30	...	...	...	2	...	1	...	...	...	...	...	33	7
	25	...	...	...	...	...	...	...	1	...	1	...	31	5
	20	...	...	...	...	...	...	...	...	1	...	...	30	9
No. 12...	60	...	1	5	4	...	...	...	...	...	...	...	3	0
	50	...	...	1	2	...	1	1	1	...	...	...	36	4
	40	...	1	...	...	1	...	...	1	1	1	...	33	5
	30	1	...	...	...	...	...	...	...	...	...	...	33	9
	25	...	1	...	1	...	...	...	...	...	...	...	31	8
	20	...	...	...	...	...	...	...	1	...	1	...	30	8

SUMMARY AND CONCLUSIONS.

The toxicity of emetine hydrochloride has been studied in rabbits, white rats, and white mice, with the result that, with the same preparation of the drug, a widely variable individual susceptibility was found in each of these species. Rowntree and Levy's work shows that the same holds true in cats and dogs. From the small amount of data at hand it seems probable that in man the range of susceptibility to emetine poisoning is fully as great as in experimental animals, and perhaps more so, in so far as it has to do with cases of amoebic dysentery, where there is always present a pathological condition, varying in intensity.

The pathological changes most often produced in experimental animals are acute degenerative changes in the parenchymatous organs.

Experiments carried out on a large number of white mice fail to show any considerable variation in the toxicity of 12 market preparations, and make it highly probable that such apparent variations are largely attributable to the widely varying individual susceptibility of animals to the drug.

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